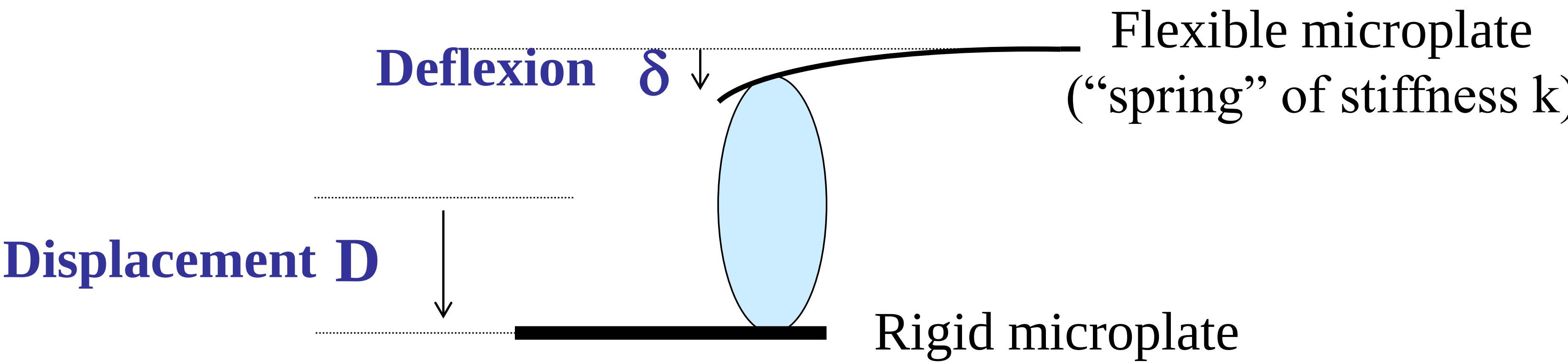
Passive properties:

Rheology of a living cell using uniaxial stretching

Uniaxial stretching

Thoumine et Ott, J. Cell Sci. ***110*** *p 2109 (1997)*

Uniaxial stretching

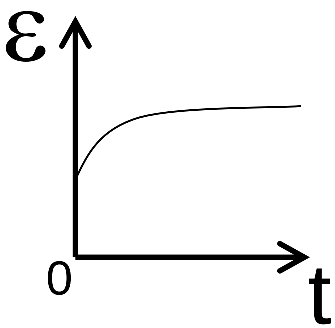


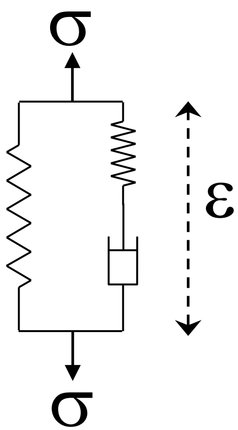
Force **$F = k\,\delta$**

Thoumine et Ott, J. Cell Sci. ***110*** *p 2109 (1997)*

Local measurements, time domain

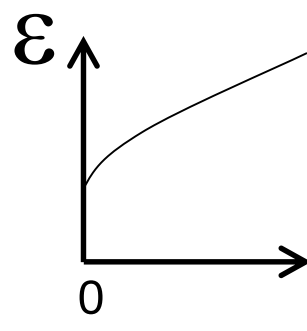
Wu et al., *Scanning*. 2003

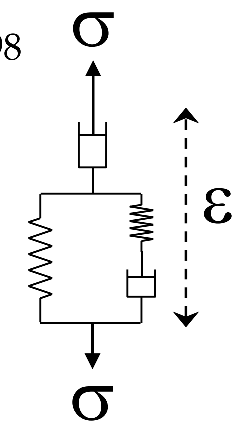




Standard linear solid

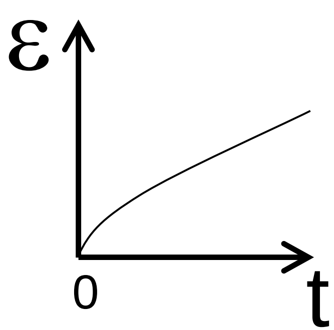
Bausch et al., *Biophys J*. 1998

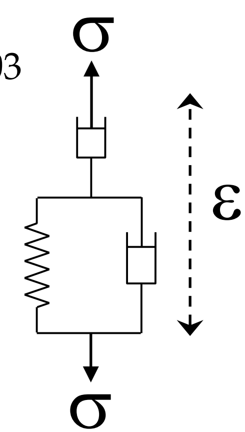




Liquid with instantaneous elasticity

Wilhelm et al., *Phys Rev E*. 2003





liquid

Step function sigma: creep

Step function epsilon: relaxation

Viscoelasticity, time domain

For a given stress input function σ(t), we obtain the resulting strain function ε(t) in three steps:

1. Obtain an expression of the Laplace transform of the stress function $\underline{\sigma}(s)$

2. Form the algebraic product: $\underline{\sigma}(s) = Y\,\underline{\varepsilon}(s)$

3. Obtain the inverse Laplace transform of the result to yield the strain function in the time domain.

The Laplace transformation is very convenient in viscoelasticity problems, because it reduces differential equations to algebraic ones

Laplace Transform

$$F(s) = \int_0^{\infty} f(t) e^{-st}\, dt$$

Laplace Transformations

Basic definition:

$$\mathcal{L}f(t) = \overline{f}(s) = \int_0^\infty f(t)\, e^{-st}\, dt$$

Fundamental properties:

$$\mathcal{L}[c_1 f_1(t) + c_2 f_2(t)] = c_2 \overline{f}_1(s) c_1 \overline{f}_2(s)$$

$$\mathcal{L}\left[\frac{\partial f}{\partial t}\right] = s\overline{f}(s) - f(0^-)$$

Some useful transform pairs:

$f(t)$	$\overline{f}(s)$
$u(t)$	$1/s$
t^n	$n!/s^{n+1}$
e^{-at}	$1/(s+a)$
$\frac{1}{a}(1-e^{-at})$	$1/s(s+a)$
$\frac{t}{a} - \frac{1}{a^2}(1-e^{-at})$	$1/s^2(s+a)$

Here $u(t)$ is the Heaviside or unit step function, defined as

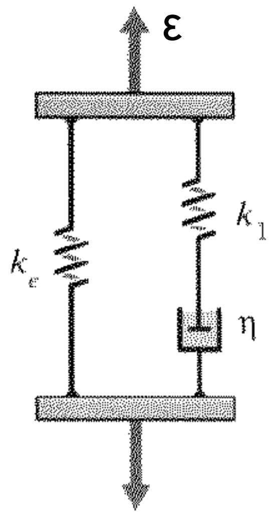
$$u(t) = \begin{cases} 0, & t < 0 \\ 1, & t \geq 0 \end{cases}$$

The convolution integral:

$$\mathcal{L}f \cdot \mathcal{L}g = \overline{f} \cdot \overline{g} = \mathcal{L}\left[\int_0^t f(t-\xi)\, g(\xi)\, d\xi\right] = \mathcal{L}\left[\int_0^t f(\xi)\, g(t-\xi)\, d\xi\right]$$

Viscoelasticity, time domain

Standard linear solid



$$\overline{\sigma} = k_e \overline{\epsilon} + \frac{k_1 s}{s + \frac{1}{\tau}} \overline{\epsilon} = \left\{ k_e + \frac{k_1 s}{s + \frac{1}{\tau}} \right\} \overline{\epsilon}$$

$$\boxed{\overline{\sigma} = \mathcal{E} \overline{\epsilon}}$$

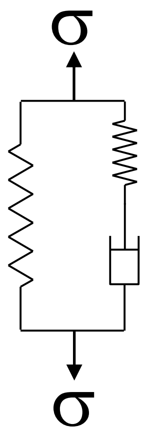
$$\mathcal{E} = k_e + \frac{k_1 s}{s + \frac{1}{\tau}}$$

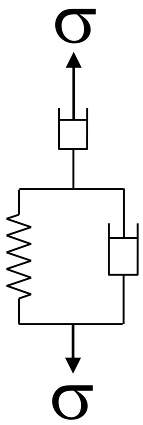
$$\epsilon(t) = \epsilon_0 u(t), \quad u(t) = \begin{cases} 0, & t < 0 \\ 1, & t \geq 0 \end{cases}$$

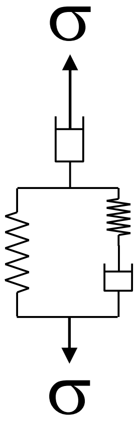
$$\overline{\epsilon} = \frac{\epsilon_0}{s} \qquad \frac{\overline{\sigma}}{\epsilon_0} = \frac{k_e}{s} + \frac{k_1}{s + \frac{1}{\tau}}$$

$$\boxed{\frac{\sigma(t)}{\epsilon_0} \equiv E_{rel}(t) = k_e + k_1 \exp(-t/\tau)}$$

Viscoelasticity, time domain



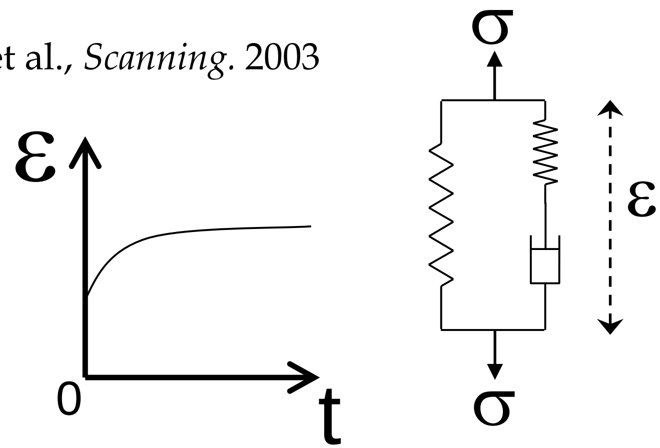




Find the solution of these problems

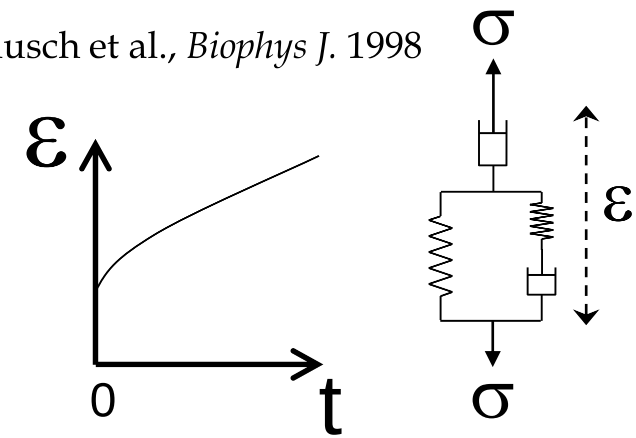
Local measurements, time domain

Wu et al., *Scanning*. 2003



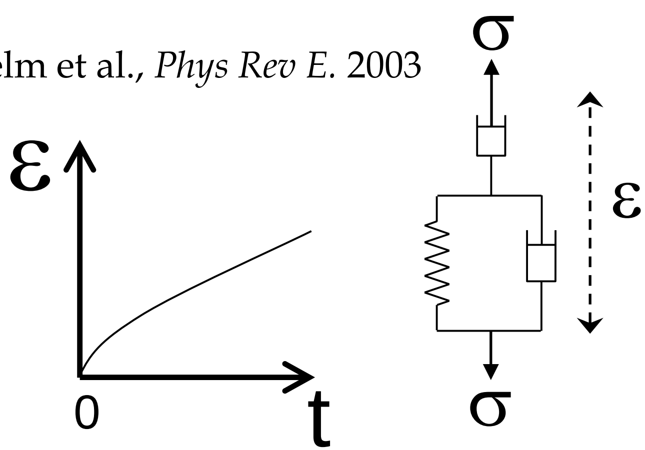
Standard linear solid

Bausch et al., *Biophys J*. 1998



Liquid with instantaneous elasticity

Wilhelm et al., *Phys Rev E*. 2003



liquid

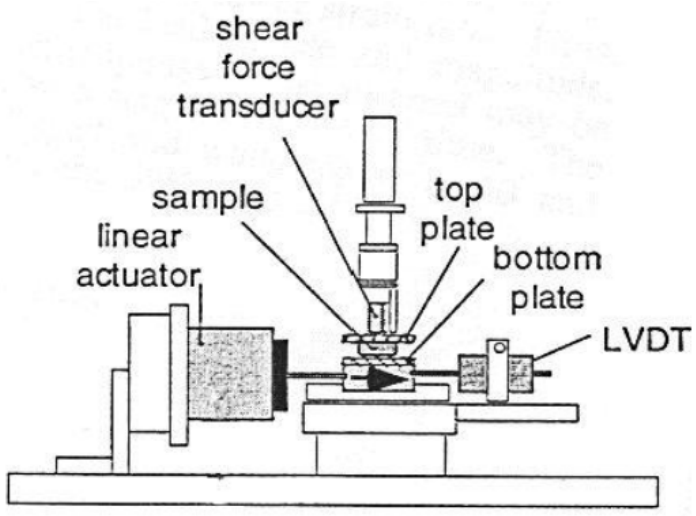
Some characteristic times

Viscous dissipation

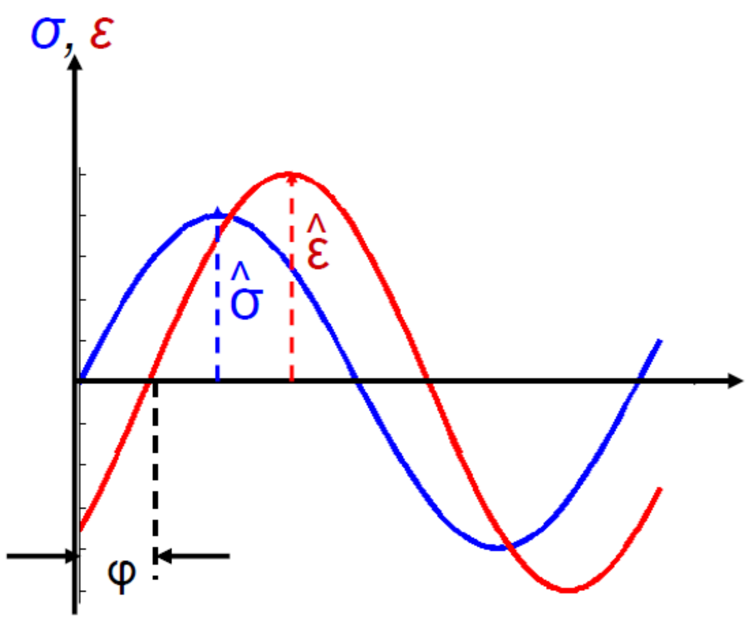
Very different behaviors

Local rheometry, frequency analysis

Creep and stress relaxation tests are convenient for studying material response at long times (minutes to days), but less accurate at shorter times (seconds and less). Dynamic tests, in which the stress (or strain) resulting from a sinusoidal strain (or stress) is measured, are often well-suited for filling out the "short-time" range of polymer response. When a viscoelastic material is subjected to a sinusoidally varying stress, a steady state will eventually be reached[2] in which the resulting strain is also sinusoidal, having the same angular frequency but retarded in phase by an angle δ; this is analogous to the delayed strain observed in creep experiments. The strain lags the stress by the phase angle δ, and this is true even if the strain rather than the stress is the controlled variable.



$$\varepsilon = \hat{\varepsilon}\sin(\omega t)$$



Local rheometry, frequency analysis

If the origin along the time axis is selected to coincide with a time at which the strain passes through its maximum, the strain and stress functions can be written as:

$$\epsilon = \epsilon_0 \cos \omega t$$

$$\sigma = \sigma_0 \cos(\omega t + \delta)$$

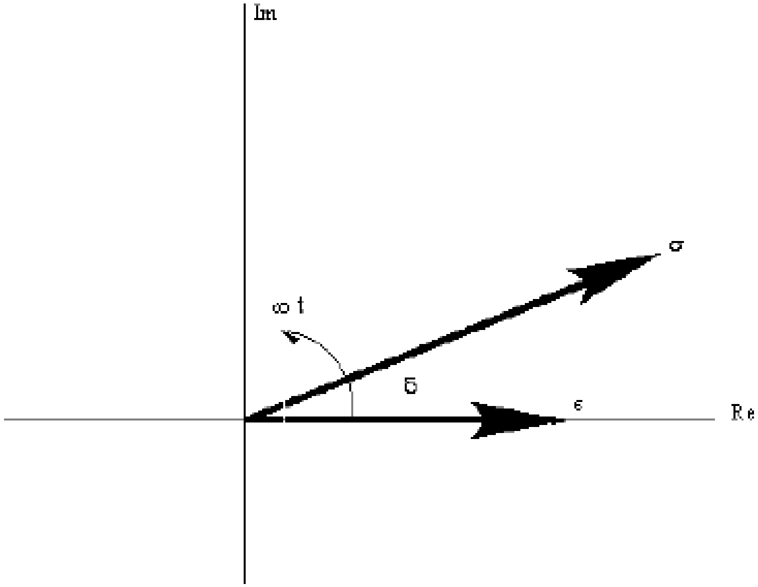
Using an algebraic maneuver common in the analysis of reactive electrical circuits and other harmonic systems, it is convenient to write the stress function as a complex quantity σ^* whose real part is in phase with the strain and whose imaginary part is 90° out of phase with it:

$$\sigma^* = \sigma_0' \cos \omega t + i\, \sigma_0'' \sin \omega t$$

Here $i = \sqrt{-1}$ and the asterisk denotes a complex quantity as usual.

Local rheometry, frequency analysis

It can be useful to visualize the observable stress and strain as the projection on the real axis of vectors rotating in the complex plane at a frequency ω. If we capture their positions just as the strain vector passes the real axis, the stress vector will be ahead of it by the phase angle δ as seen in Fig. 7.



Local rheometry, frequency analysis

Figure 7 makes it easy to develop the relations between the various parameters in harmonic relations:

$$\tan\delta = \sigma_0''/\sigma_0'$$
$$|\sigma^*| = \sigma_0 = \sqrt{(\sigma_0')^2 + (\sigma_0'')^2}$$
$$\sigma_0' = \sigma_0 \cos\delta$$
$$\sigma_0'' = \sigma_0 \sin\delta$$

We can use this complex form of the stress function to define two different dynamic moduli, both being ratios of stress to strain as usual but having very different molecular interpretations and macroscopic consequences. The first of these is the "real," or "storage," modulus, defined as the ratio of the in-phase stress to the strain:

$$E' = \sigma_0'/\epsilon_0$$

The other is the "imaginary," or "loss," modulus, defined as the ratio of the out-of-phase stress to the strain:

$$E'' = \sigma_0''/\epsilon_0$$

The terms "storage" and "loss" can be understood more readily by considering the mechanical work done per loading cycle. The quantity $\int \sigma\, d\epsilon$ is the strain energy per unit volume (since σ = force/area and ϵ = distance/length). Integrating the in-phase and out-of-phase components separately:

$$W = \oint \sigma d\epsilon = \oint \sigma \frac{d\epsilon}{dt} dt$$

$$= \int_0^{2\pi/\omega} (\sigma_0' \cos \omega t)(-\epsilon_0 \omega \sin \omega t) dt + \int_0^{2\pi/\omega} (\sigma_0'' \sin \omega t)(-\epsilon_0 \omega \sin \omega t) dt$$

$$= 0 - \pi \sigma_0'' \epsilon_0$$

Note that the in-phase components produce no net work when integrated over a cycle, while the out-of-phase components result in a net dissipation per cycle equal to:

$$W_{dis} = \pi \sigma_0'' \epsilon_0 = \pi \sigma_0 \epsilon_0 \sin \delta$$

This should be interpreted to illustrate that the strain energy associated with the in-phase stress and strain is reversible; i.e. that energy which is stored in the material during a loading cycle can be recovered without loss during unloading. Conversely, energy supplied to the material by the out-of-phase components is converted irreversibly to heat.

The maximum energy stored by the in-phase components occurs at the quarter-cycle point, and this maximum stored energy is:

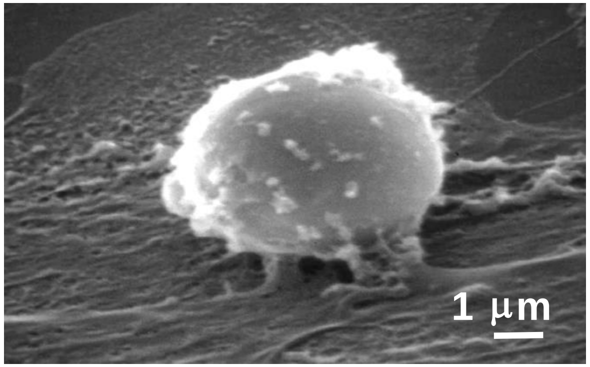
$$W_{st} = \int_0^{\pi/2\omega} (\sigma_0' \cos \omega t)(-\epsilon_0 \omega \sin \omega t) dt$$

$$= -\frac{1}{2} \sigma_0' \epsilon_0 = -\frac{1}{2} \sigma_0 \epsilon_0 \cos \delta$$

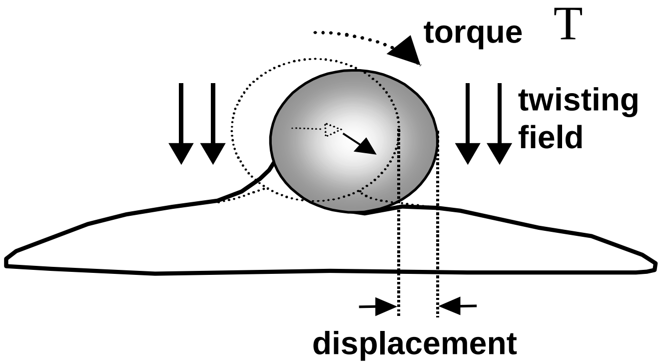
The relative dissipation – the ratio of W_{dis}/W_{st} – is then related to the phase angle by:

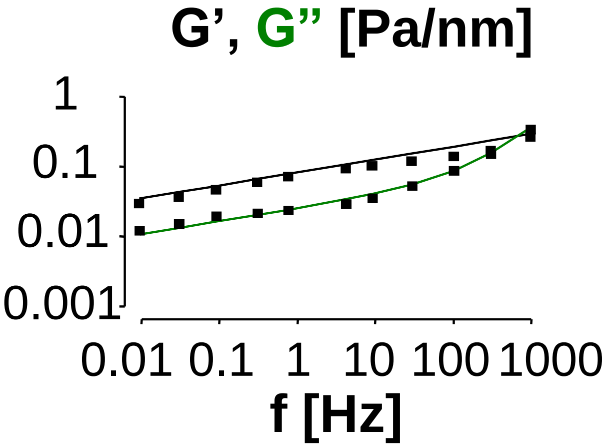
$$\frac{W_{dis}}{W_{st}} = 2\pi \tan \delta$$

Local rheometry, frequency analysis



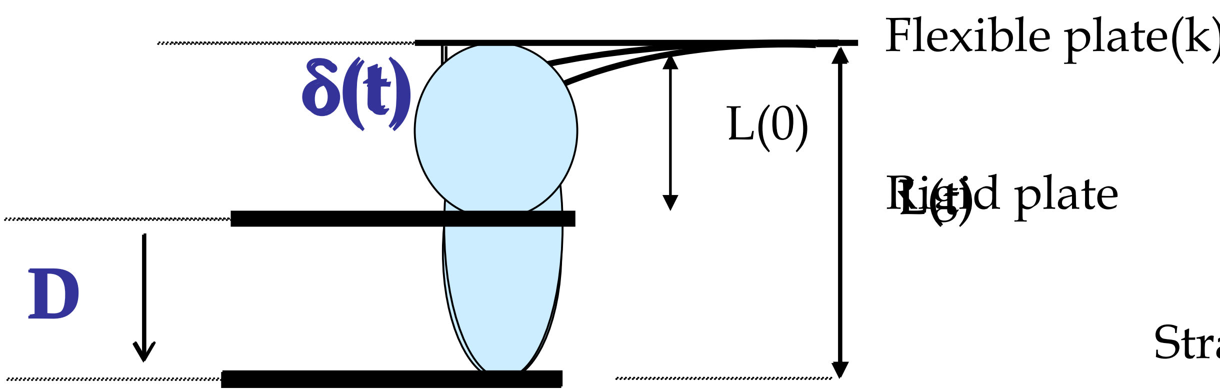
Fabry et al., *Phys Rev Lett.* 2001





1. From uniaxial stretching to single cell rheometer

Uniaxial stretching $(\dot{\sigma} \neq 0 \text{ et } \dot{\varepsilon} \neq 0)$



Stress :

$$\sigma(t) = \frac{F(t)}{S}$$

$$F(t) = k.\delta(t)$$

Strain :

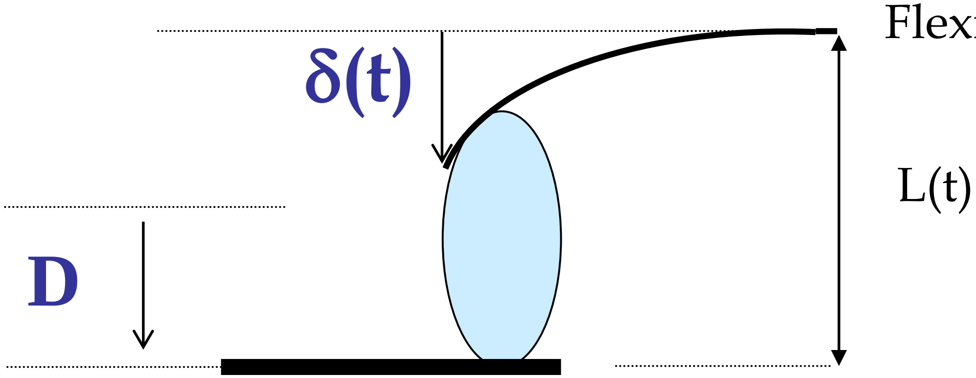
$$\varepsilon(t) = \frac{\mathrm{L(t)} - \mathrm{L(0)}}{\mathrm{L(0)}}$$

- The strain response to an arbitrary stress history is obtained from J(t) by *superposition*

$$\varepsilon(t) = \int_0^t J(t-\tau)\,d\sigma = \int_0^t J(t-\tau)\frac{d\sigma}{d\tau}\,d\tau$$

1. From uniaxial stretching to single cell rheometer

Uniaxial stretching $(\dot{\sigma} \neq 0 \text{ et } \dot{\varepsilon} \neq 0)$



Stress :

$$\sigma(t) = \frac{F(t)}{S}$$

$$F(t) = k.\delta(t)$$

Strain :

$$\varepsilon(t) = \frac{\mathrm{L(t)} - \mathrm{L(0)}}{\mathrm{L(0)}}$$

Stress-strain relationship

$$\varepsilon(t) = J(t)\sigma(0) + \int_0^{+\infty} J(t-t')\dot{\sigma}(t')dt'$$

$\dot{\sigma} \neq 0 \Rightarrow$ Very difficult to determine J

Avoid convolution product ⇔ oscillations (σ(ω))

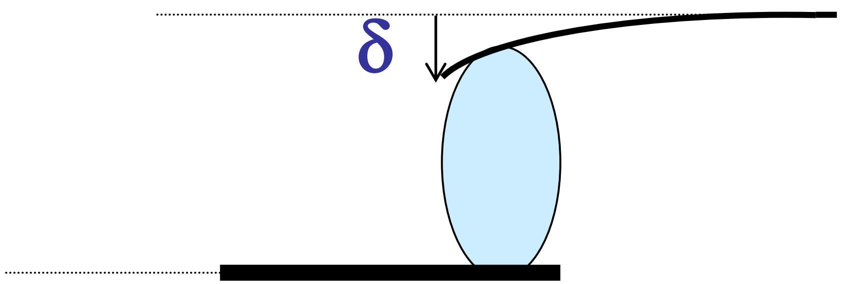
ou constant stress $(\dot{\sigma} = 0)$

1. From uniaxial stretching to single cell rheometer

Rheometer $(\dot{\sigma} = 0)$

$$\varepsilon(t) = J(t)\sigma(0)$$

At constant stress: measurement of J ⇔ mesurement of strain ε



F = k.δ

$\dot{\sigma} = 0$ ⇔ δ **constant**

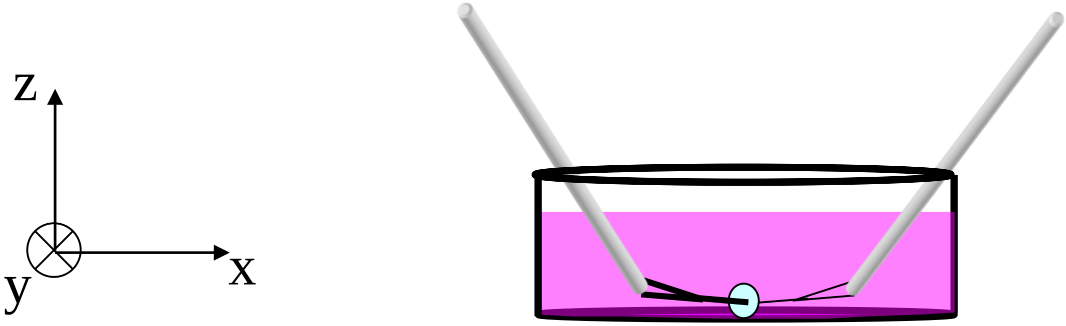
$$\delta = 10\mu m$$

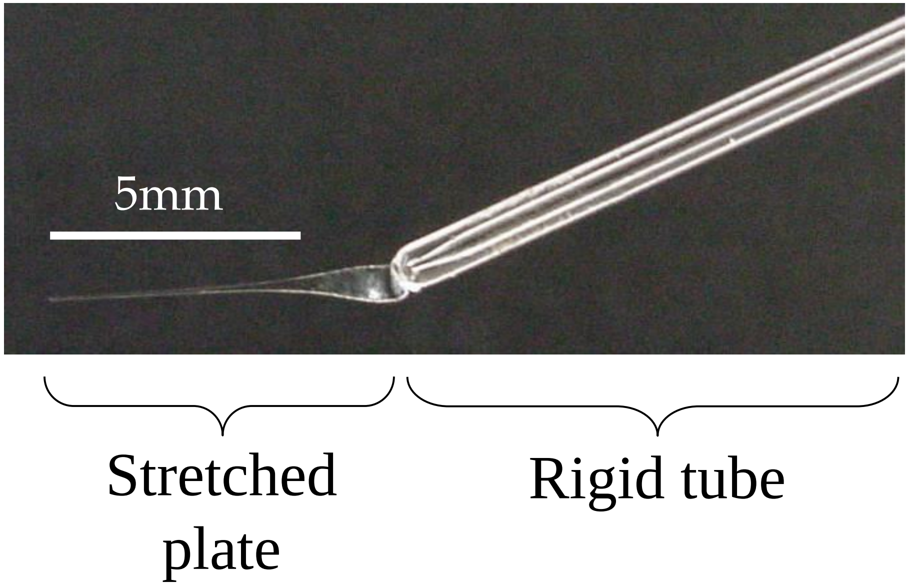
$$O,O3nN.\mu m^{-1} < k < 15nN.\mu m^{-1}$$

$$300pN < F < 1\mu N$$

Single cell rheometer

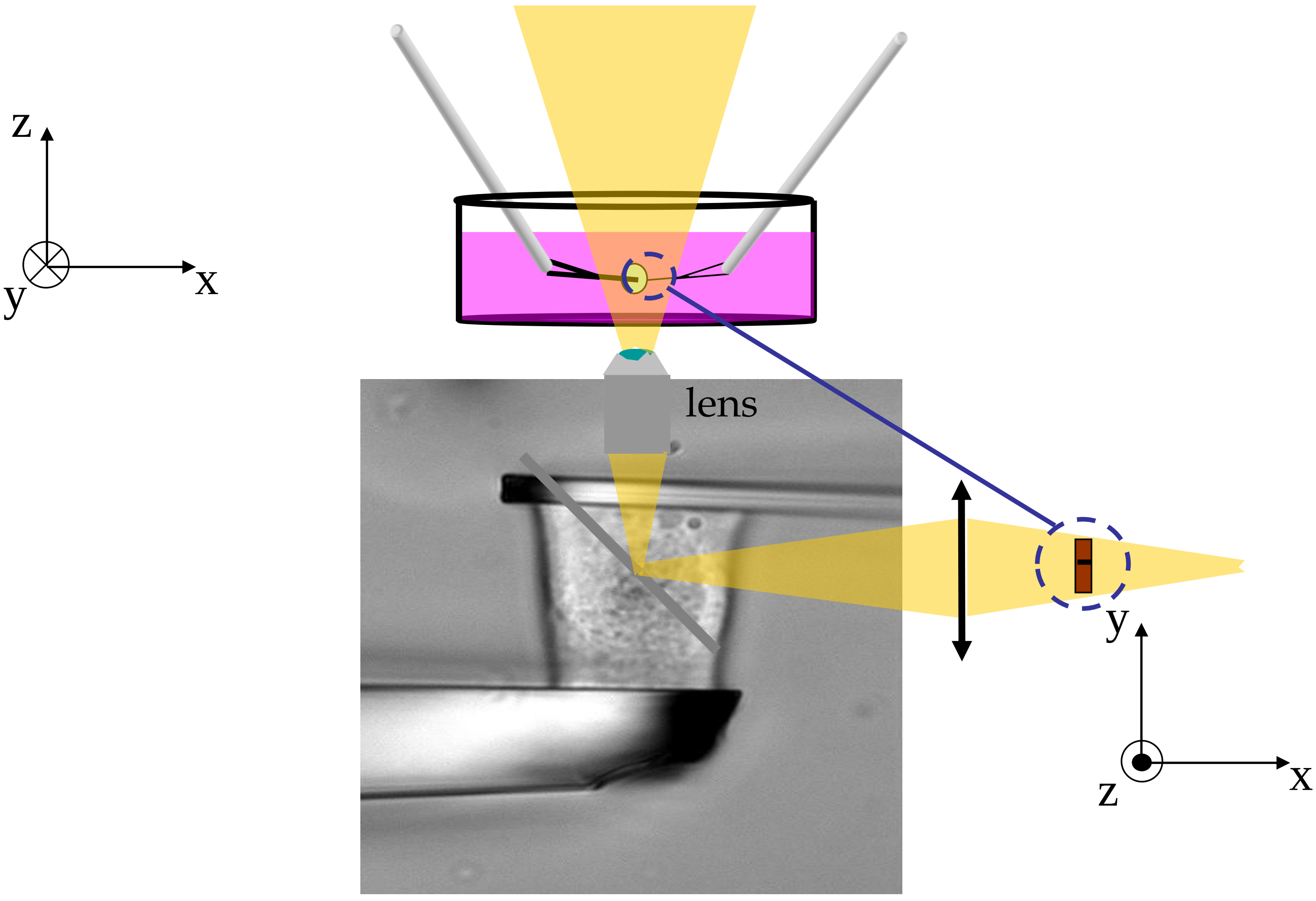
Desprat et al., Rev.Sci. Instrum. ***77****, 055111-1 (2006)*



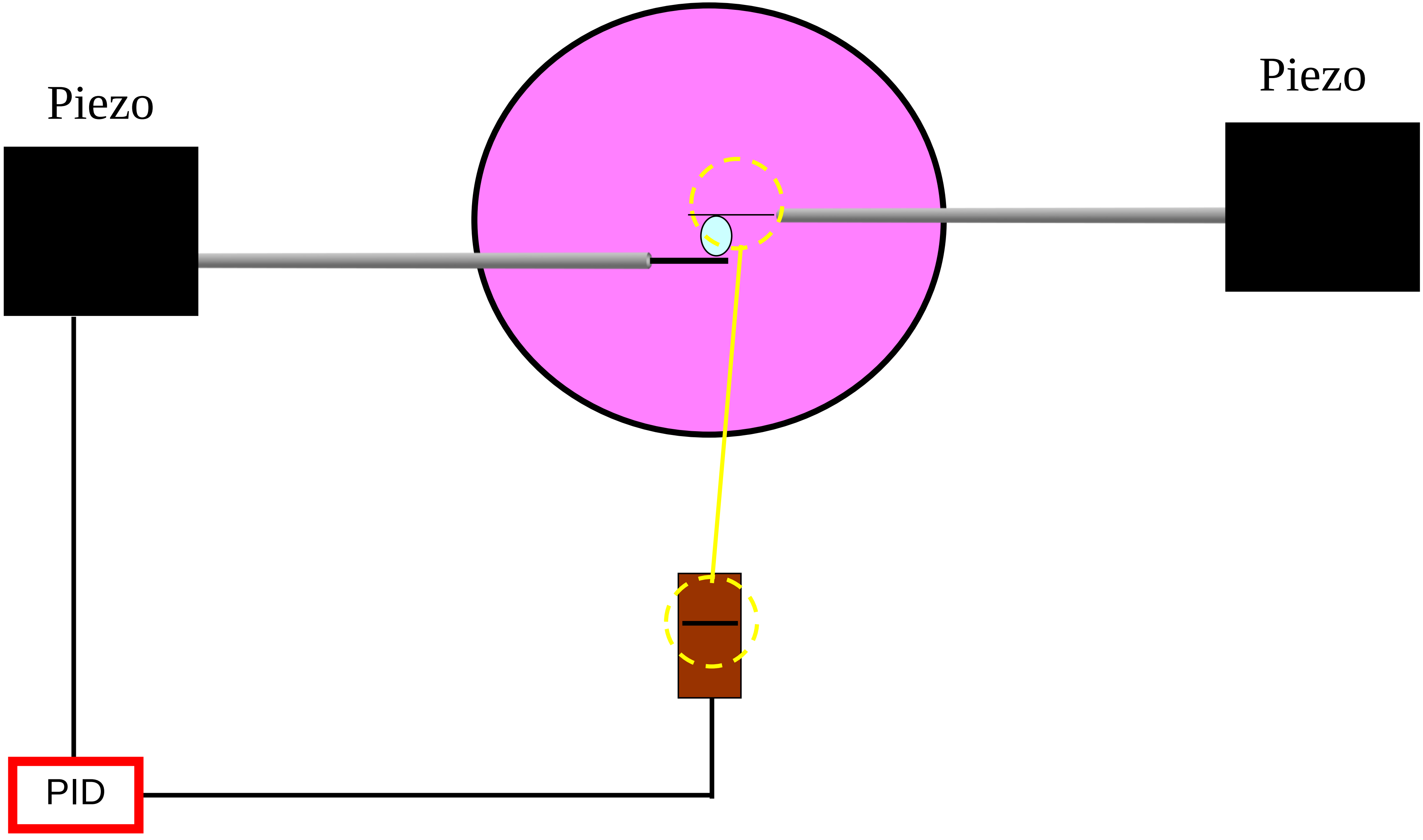


Single cell rheometer

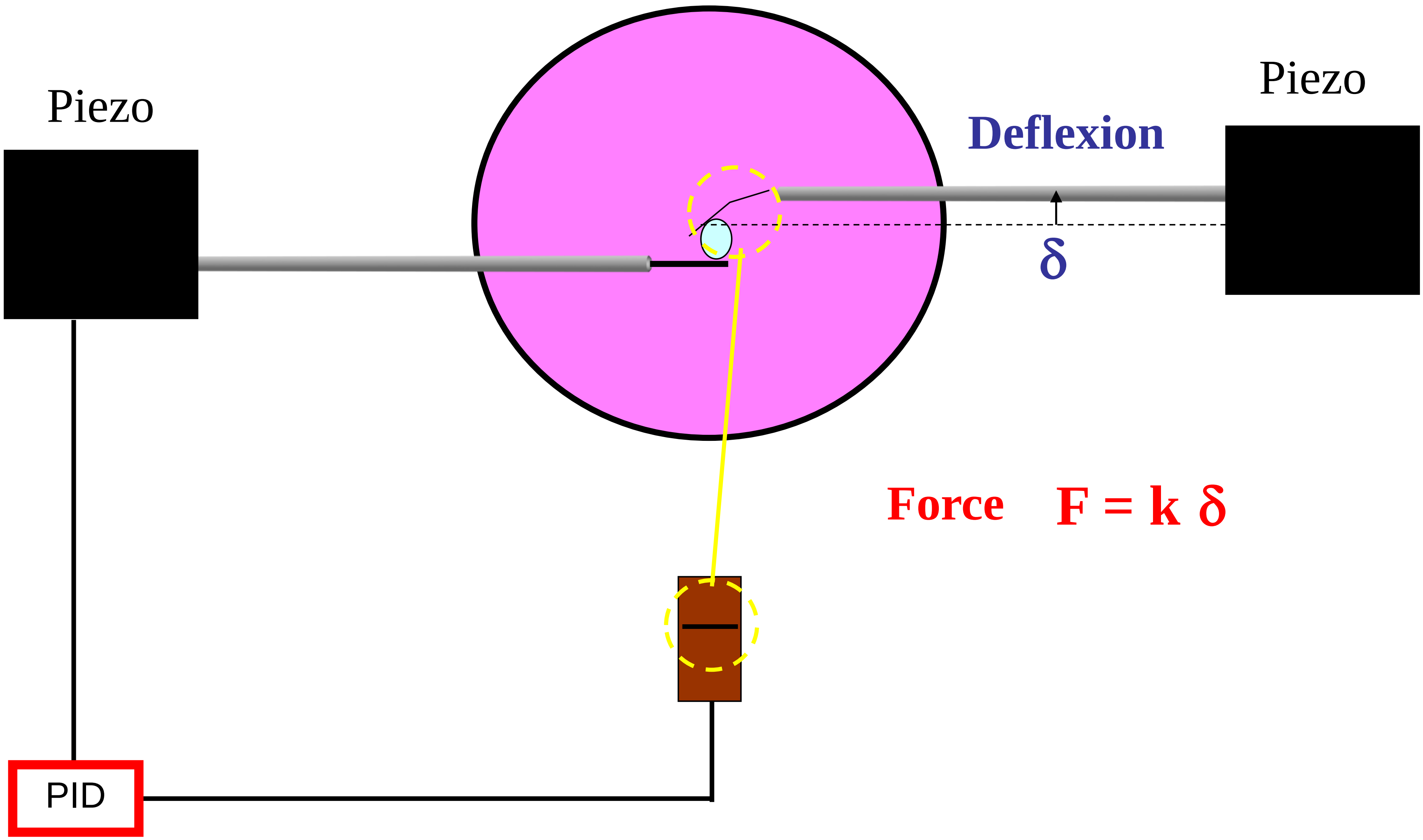
Desprat et al., Rev.Sci. Instrum. ***77****, 055111-1 (2006)*



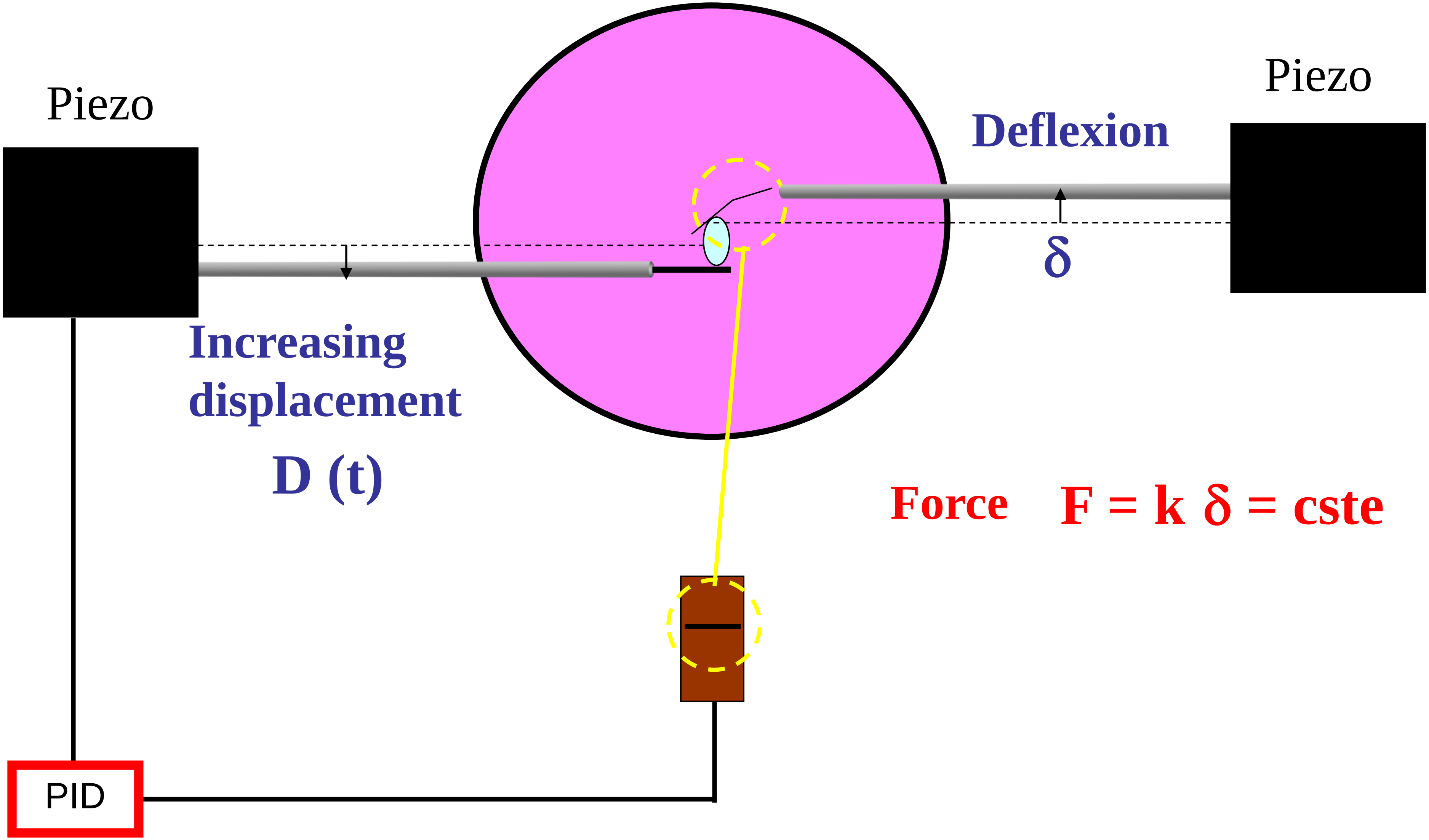
Creep experiment



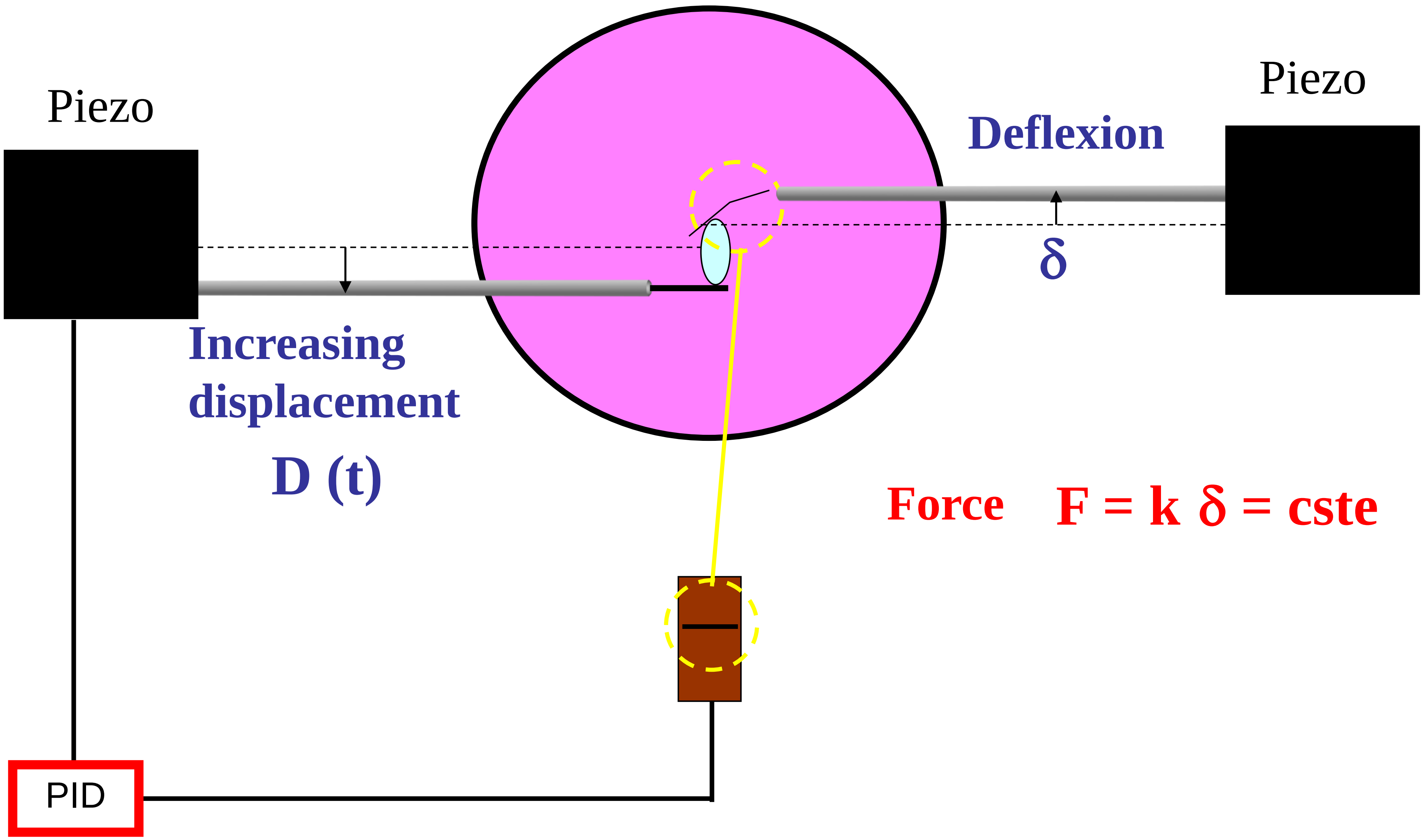
Creep experiment



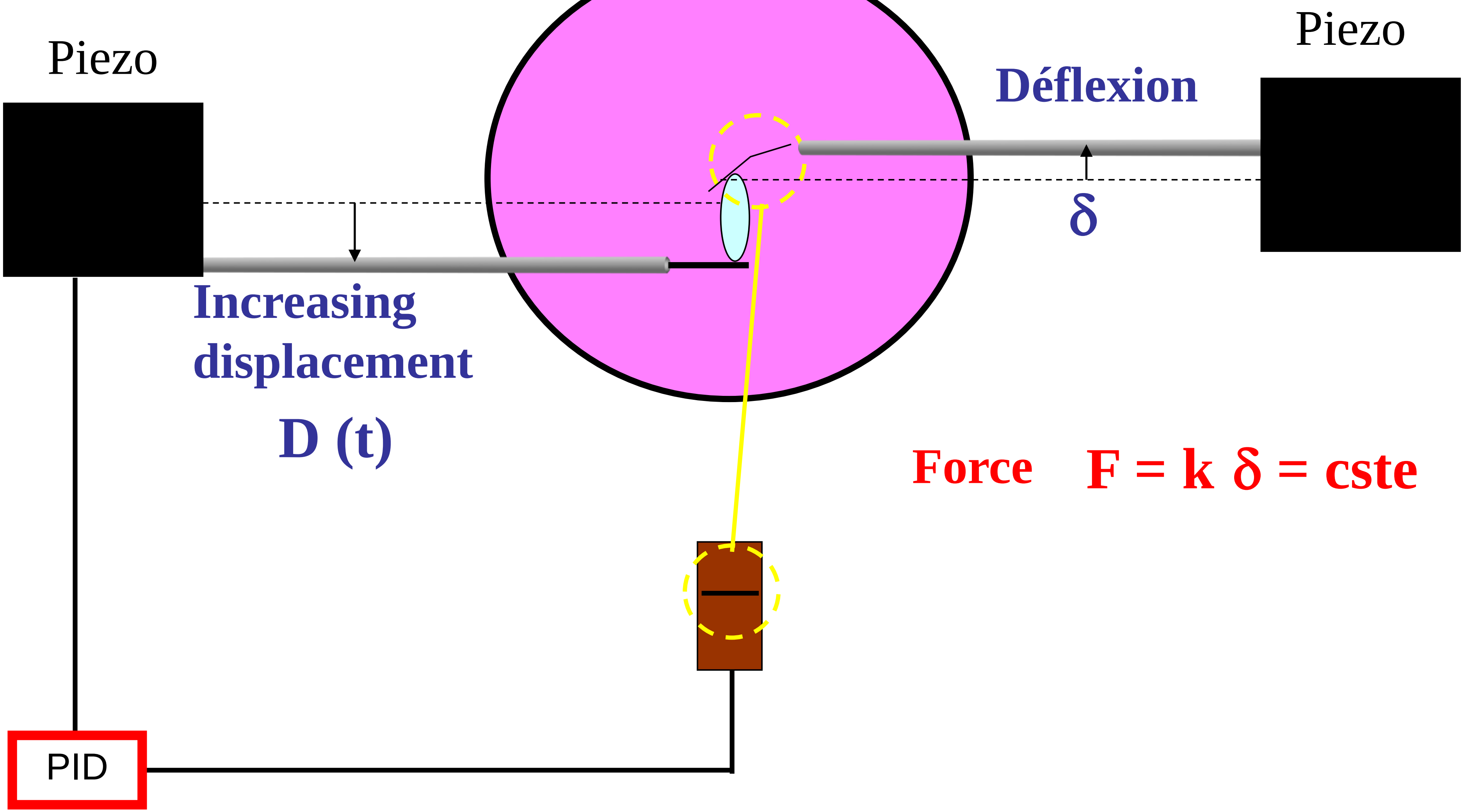
Creep experiment



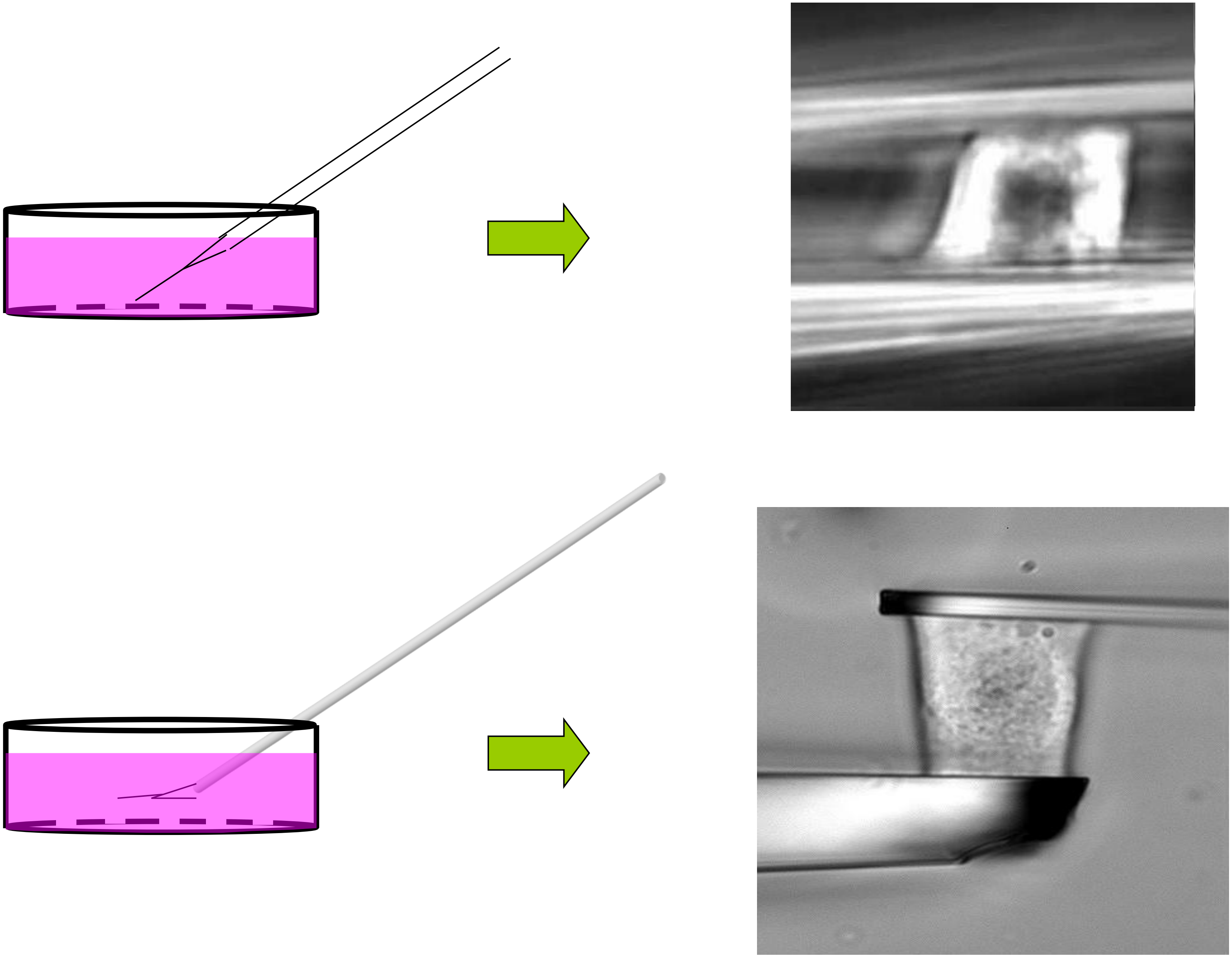
Creep experiment



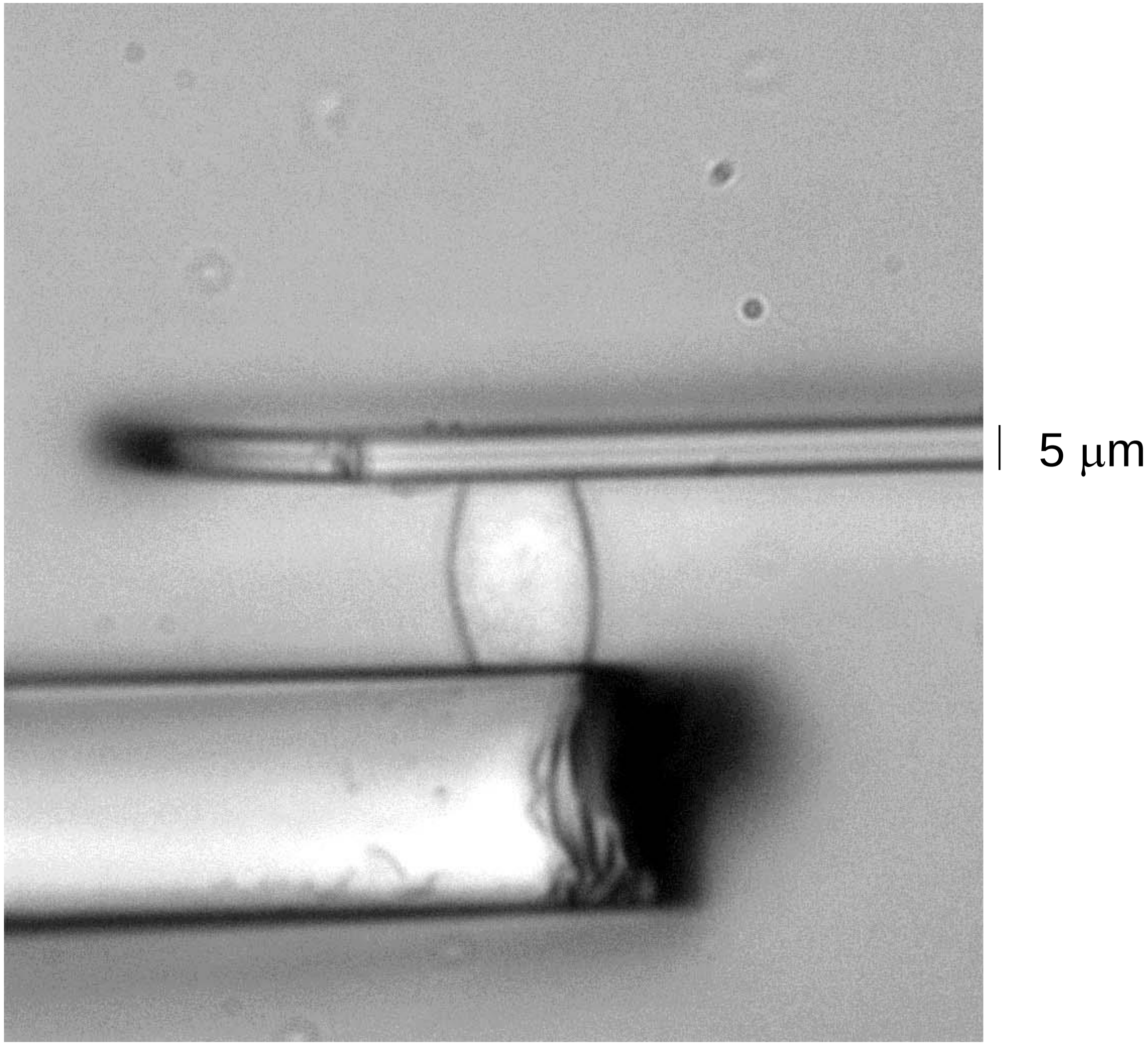
Creep experiment



Cell stretching D(t) at constant force

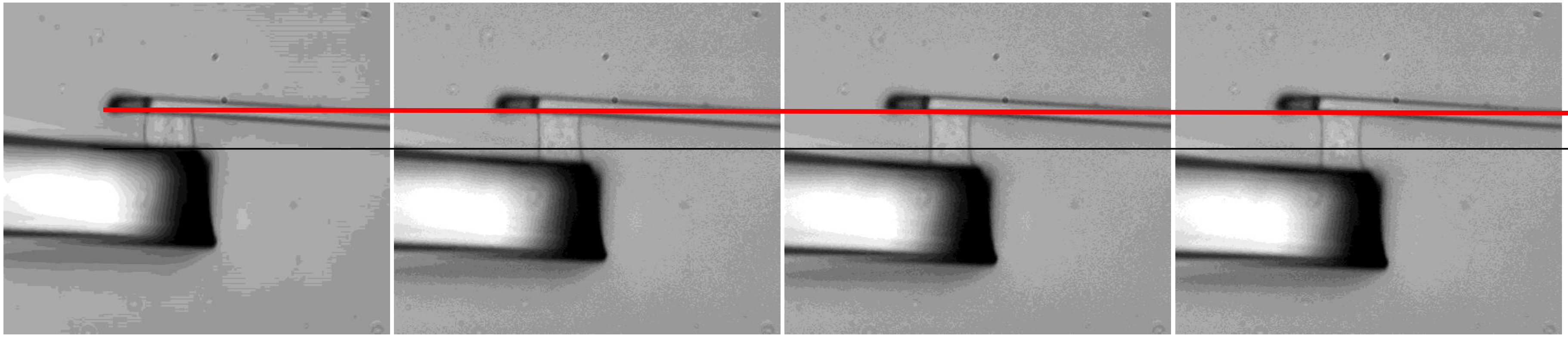


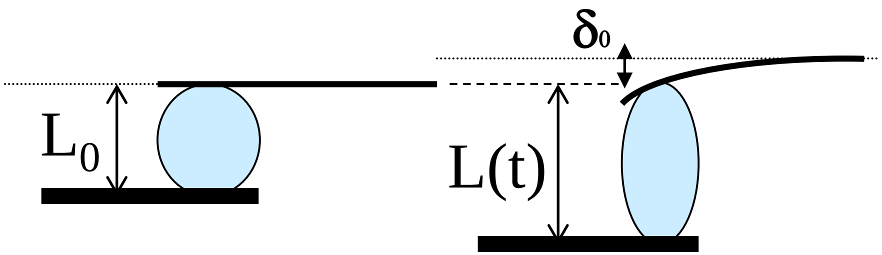
Creep experiment



Plates treated with Glutaraldehyde, non specific adhesion

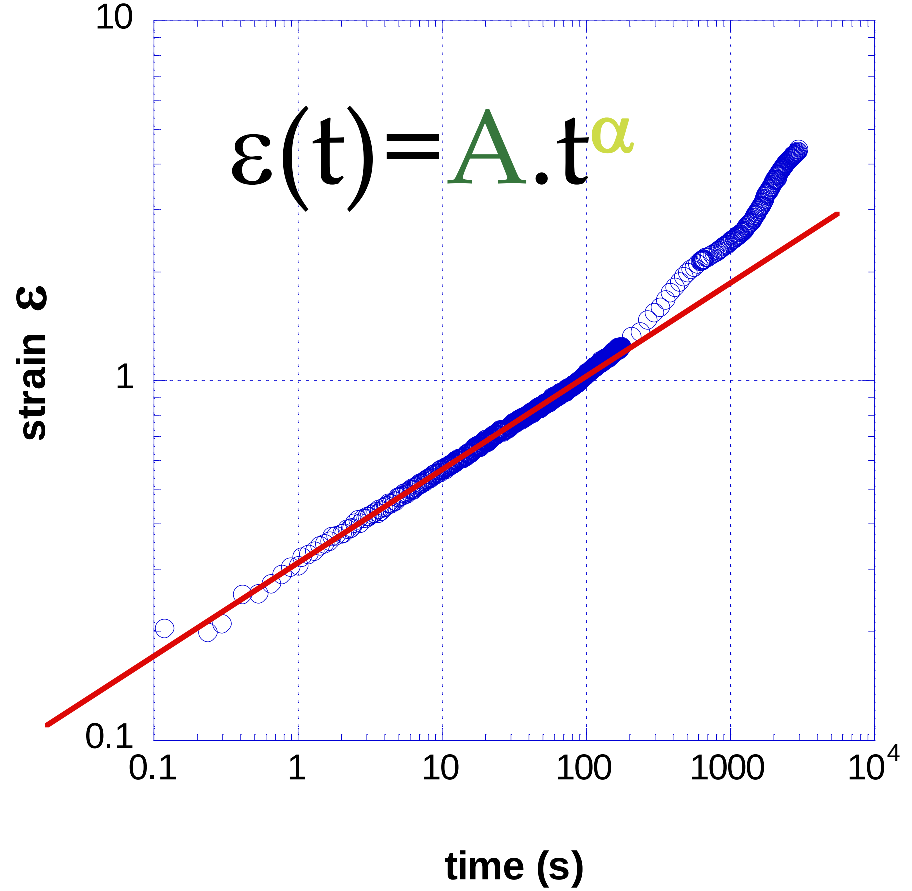
Creep experiment





Force $F_0 = k\ \delta_0 = cst$

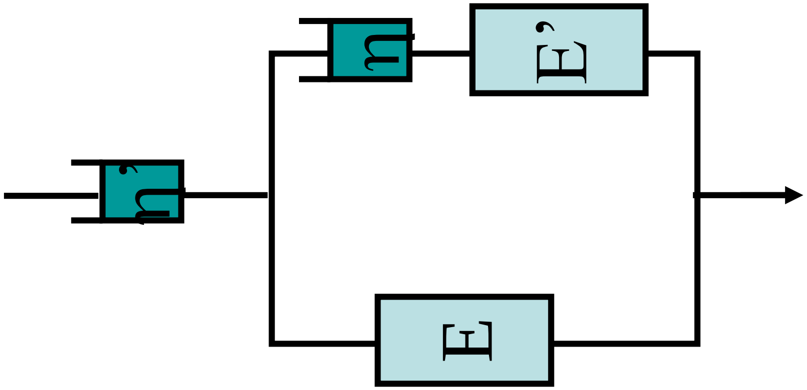
Strain $\varepsilon(t) = \dfrac{L(t) - L_0}{L_0}$

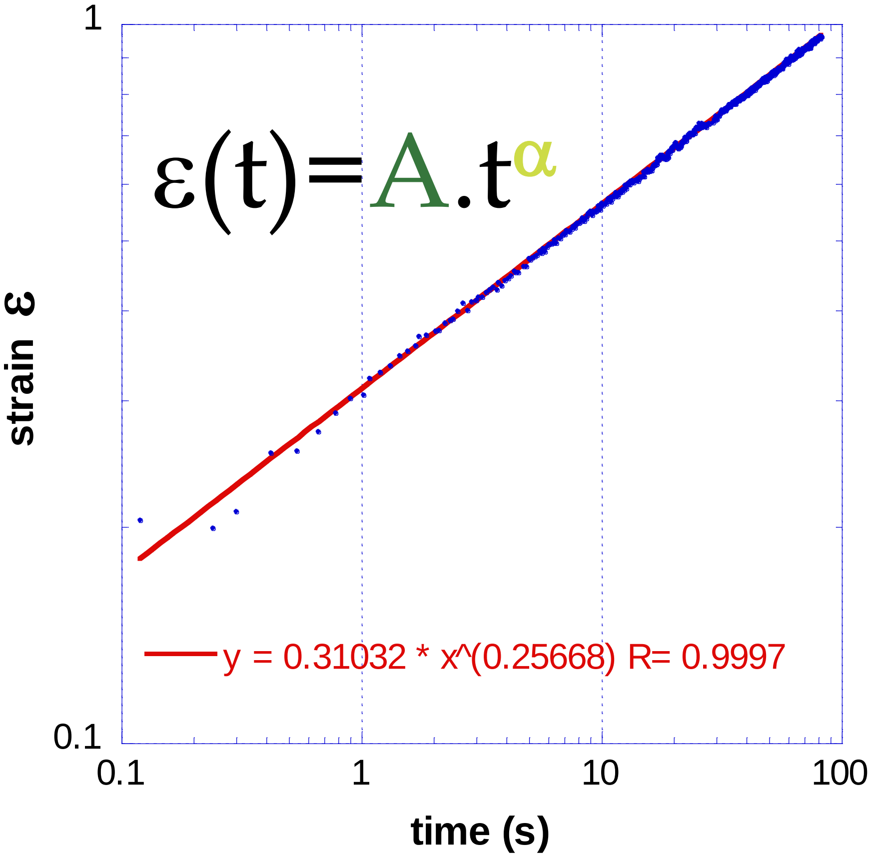


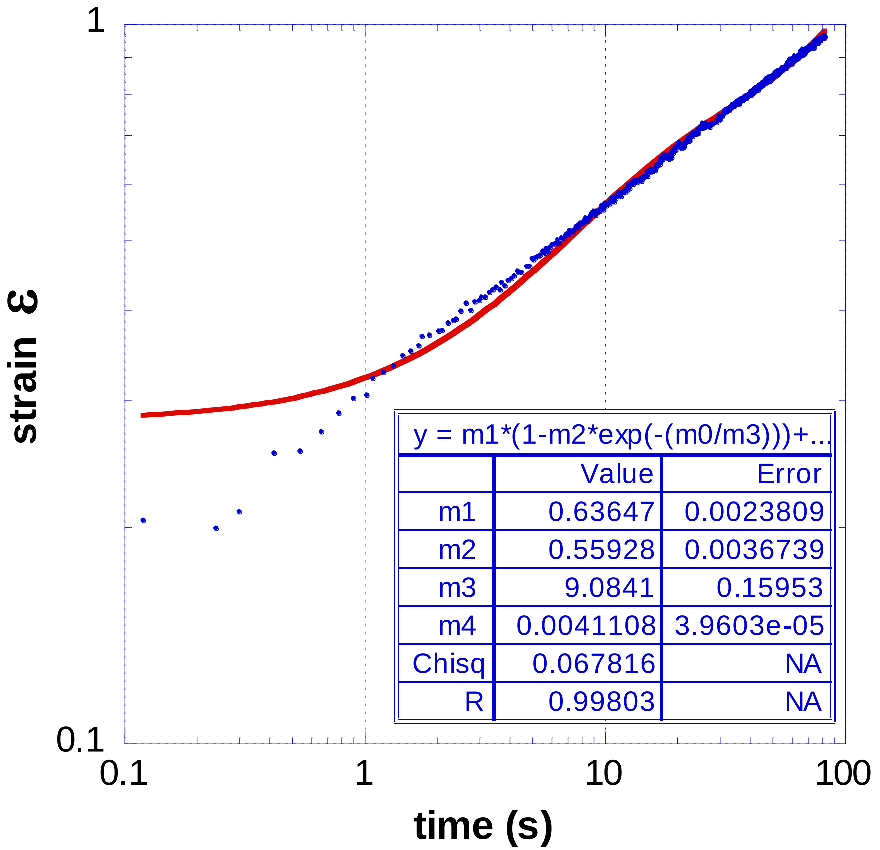
No characteristic time

Comparison with simple mechanical models

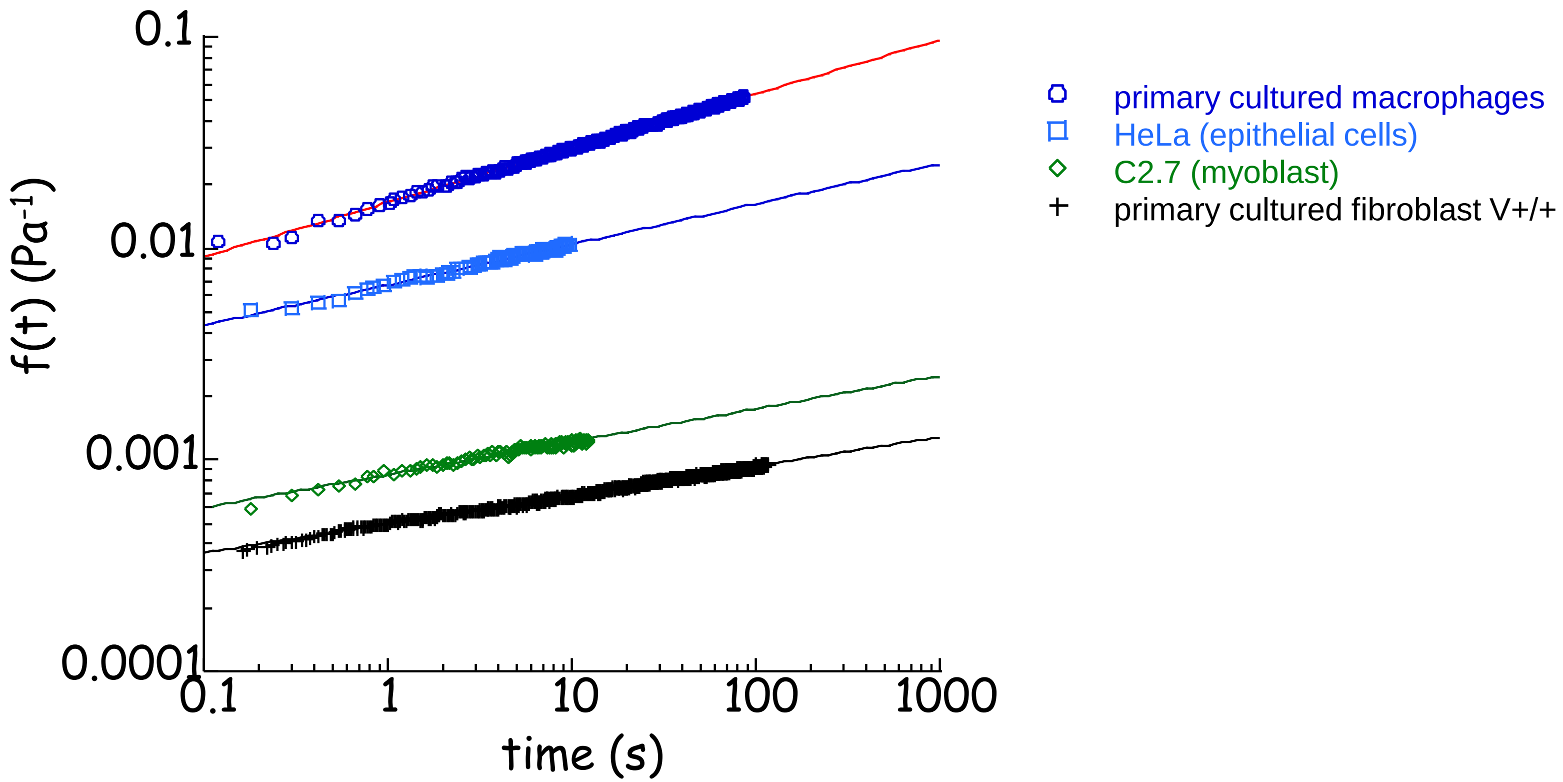
No characteristic time







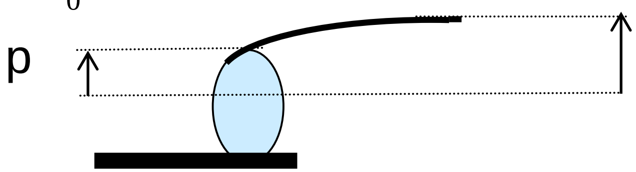
« Universal« behavior



cancer cells F9, J774 alveolar macrophages, A549 alveolar epithelial cells, BEAS-2B of bronchi, human neutrophiles

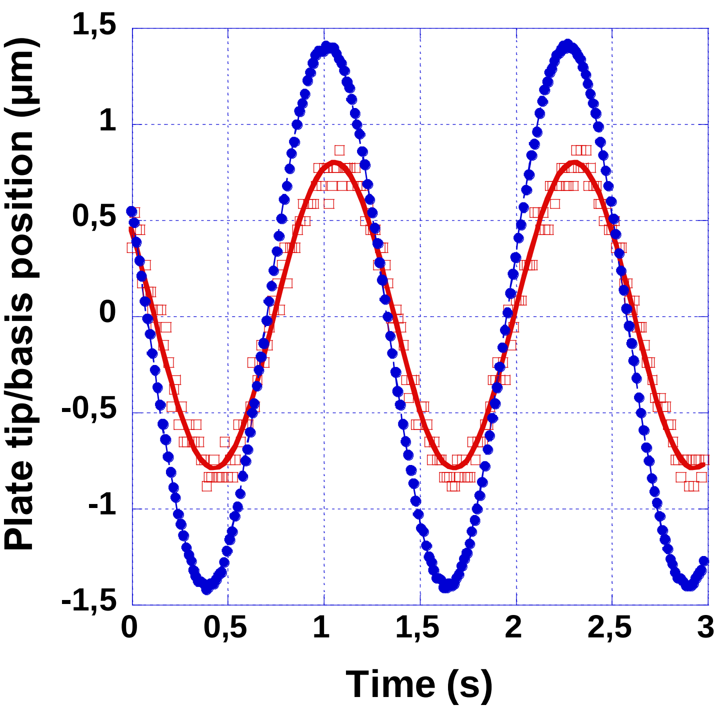
Viscoelastic modulus at small strains

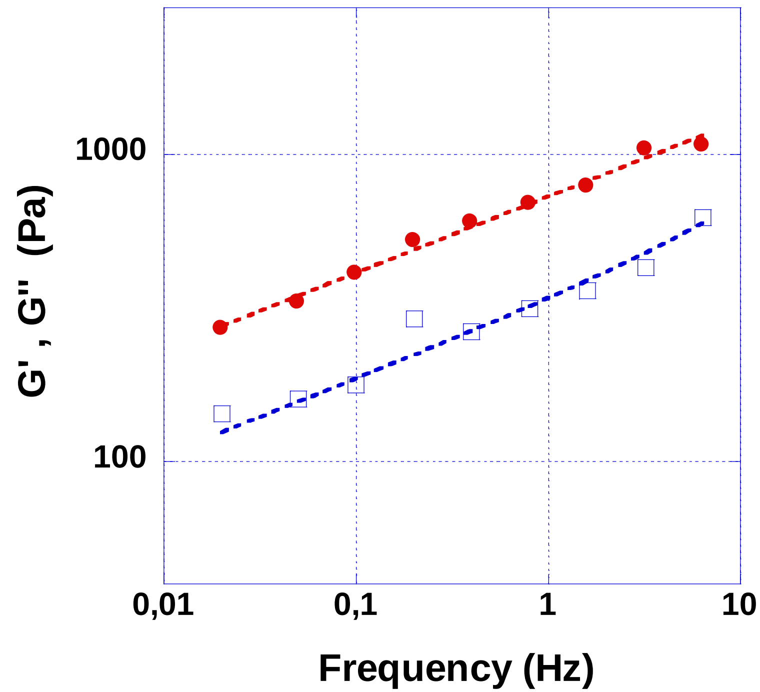
$$\tilde{\varepsilon}(\omega) = \varepsilon_0 e^{i(\omega t + \varphi)}$$



$$\delta(t) = \delta_\circ \sin(\omega t)$$

$$\tilde{\sigma}(\omega) = \sigma_0 e^{i\omega t}$$





Power law behavior is consistent
Linearity at large strains

Desprat et al., Rev.Sci. Instrum. ***77****, 055111-1 (2006)*

Derivations

The fundamental relation of **linear viscoelasticity**

$$\varepsilon(t) = J(t)\sigma(0) + \int_0^t J(t-t')\dot{\sigma}(t')dt'$$

Then becomes

$$\varepsilon(t) = J(t)\sigma(0) + \int_0^t J(t-t')\sigma(0)\dot{\varepsilon}(t')dt'$$

Laplace transform then yelds

$$F(p) = \mathcal{L}\{f(t)\} = \int_{0^-}^{+\infty} e^{-pt} f(t)\,dt.$$

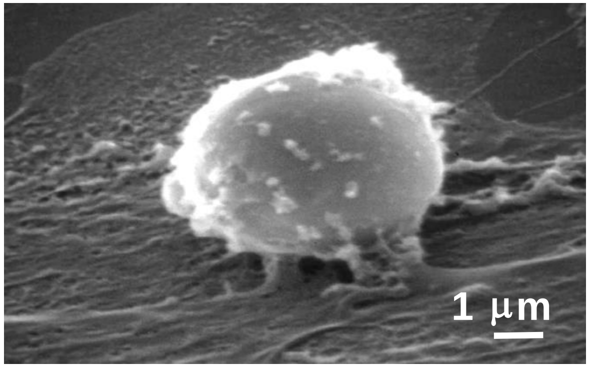
$$\tilde{\varepsilon}(s) = \frac{\sigma(0)\tilde{J}(s)}{[1-s\sigma(0)\tilde{J}(s)]}$$

Assuming that $J(t) = At^{\alpha}$ **as measured in the creep regime**, one finds

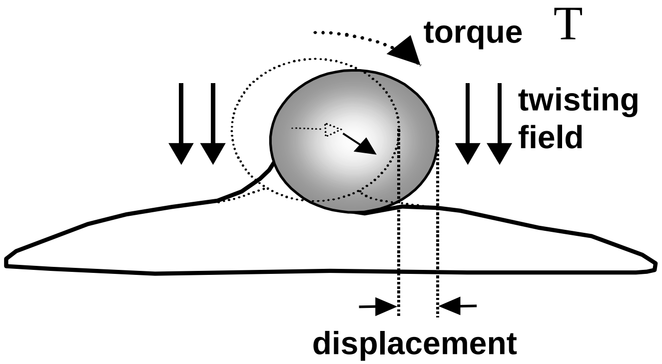
$$\varepsilon(t) = \sum_{n=1}^{+\infty} \frac{[\Gamma(1+\alpha)\sigma(0)\mathrm{A}t^{\alpha}]^n}{\Gamma(1+n\alpha)}$$

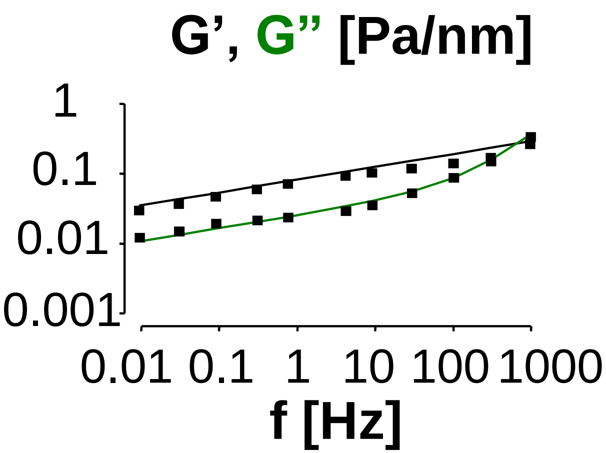
Thus, at high strains, deformation should well be described by a sum of integer powers of the creep function *J(t)*

Local rheometry, frequency analysis



Fabry et al., *Phys Rev Lett.* 2001





No characteristic time

Elasticity and dissipation from same origin

Unique behavior preserved

AVERAGE !

$G^*(\omega) = G_0\Gamma(2\text{-}x)(j\,\omega\tau_0)^{x-1} + j\omega\mu$

$\Gamma : z \mapsto \int_0^{+\infty} t^{z-1}\, e^{-t}\, \mathrm{d}t \qquad \Gamma(z+1) = z\,\Gamma(z).$

Soft glassy medium behavior

- Out of balance
- Structural disorder
- Metastability
- Effective temperature (glass transition)

Soft Glassy Material or … Fractal Gel

AFM: **L~30 nm** $\alpha \sim 0{,}20$; $G_0 \sim 710$ Pa (*Alcaraz et al., Biophys J., 2003*)

MTC; OT: **L~3 µm** $\alpha \sim 0{,}20$; $G_0 \sim 300$ à 3000 Pa (*Fabry et al., Phys Rev E., 2003*)

(*Balland et al., E. Biophys. J., 2005*)

In agreement with measurements at the cellular scale L~30 µm

$$J(t) = A.t^{\alpha} \xrightarrow{\text{T.F}} G'(f) = \frac{(2\pi)^{\alpha}\cos(\alpha\frac{\pi}{2})}{A\Gamma(1+\alpha)} f^{\alpha}$$

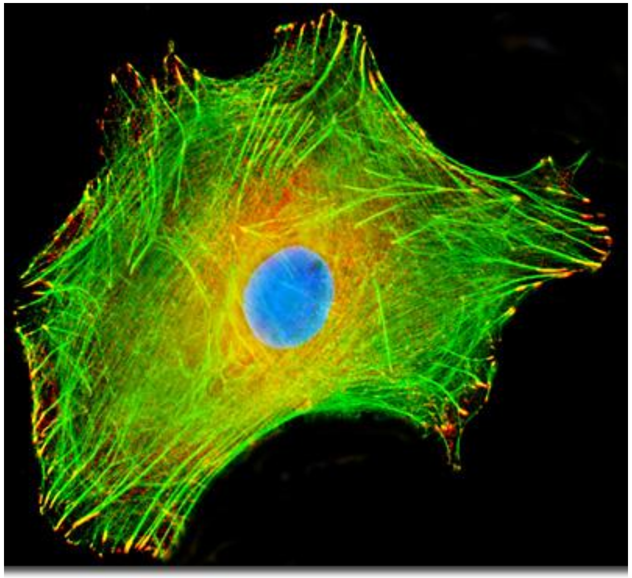
$G_0 = 660$ Pa

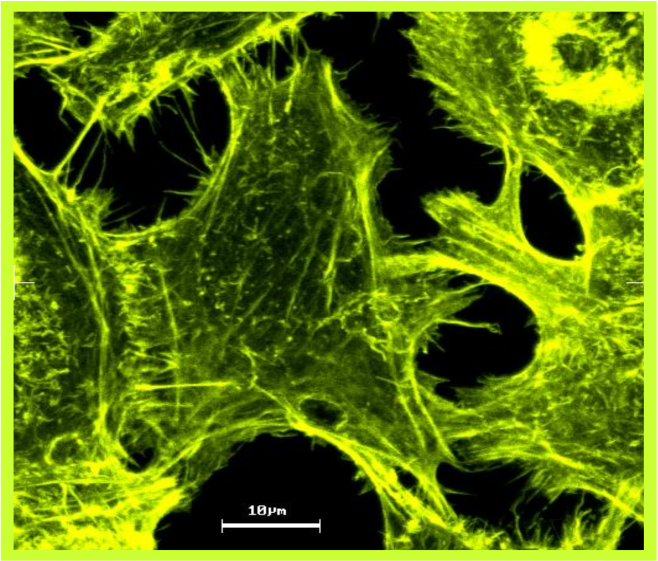
Auto-similarity ?

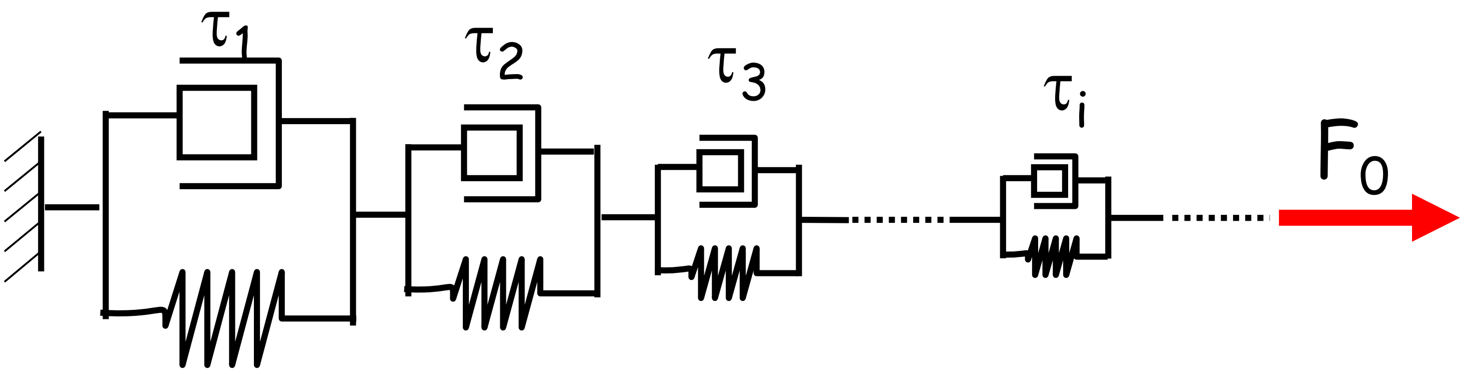
A simple constitutive model

Actine network:
- individual filaments
- bundles
- fibers

unevenly distributed in the cell body







The actin network is modeled by an infinite series of nested elementary viscoelastic units with a wide distribution $p(\tau)$ relaxation times τ

Distribution of response times

*Balland et al., Phys.Rev.E **74**, 021911 (2006)*

Simple assumptions:

- N(d) number of units of size d
  $N(d) \sim d^{-a}$ if self similar structure

- relaxation time linked to spatial scale: $\tau \sim d^{b}$

Then **$p(\tau) \sim \tau^{\alpha-2}$** with $\alpha = 1 - a/b$

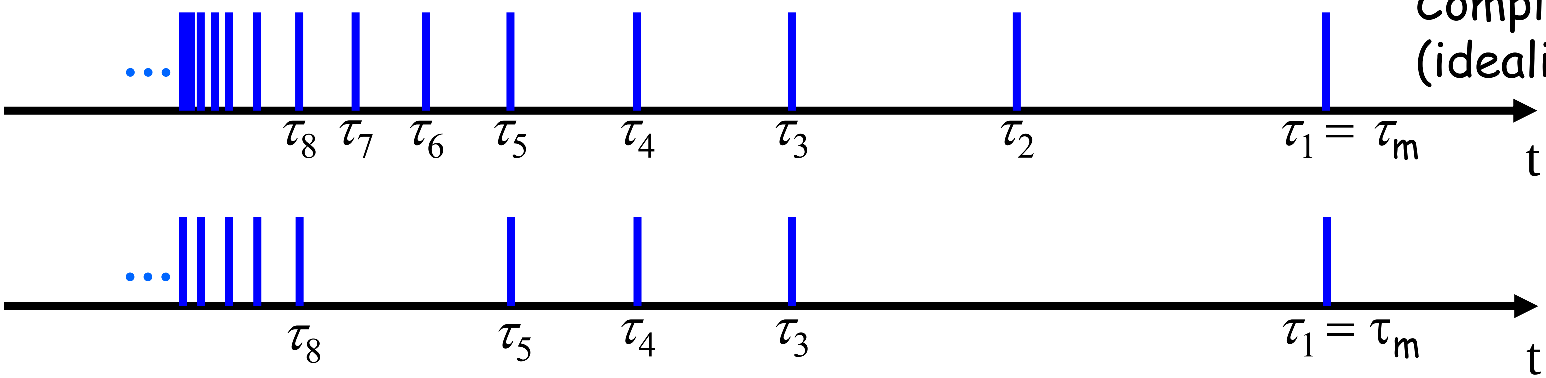
$p(\tau) \sim \tau^{\alpha-2}$ in power law ➡ creep function J(t) as well

$$\frac{dJ}{dt} = \sum_{i=1}^{\infty} \exp(-\frac{t}{\tau_i}) \approx \int_0^{\infty} \tau^{\alpha-2} \exp(-\frac{t}{\tau}) d\tau \propto t^{\alpha-1}$$

so **$J(t) \sim t^{\alpha}$**

Agreement with experimental observations

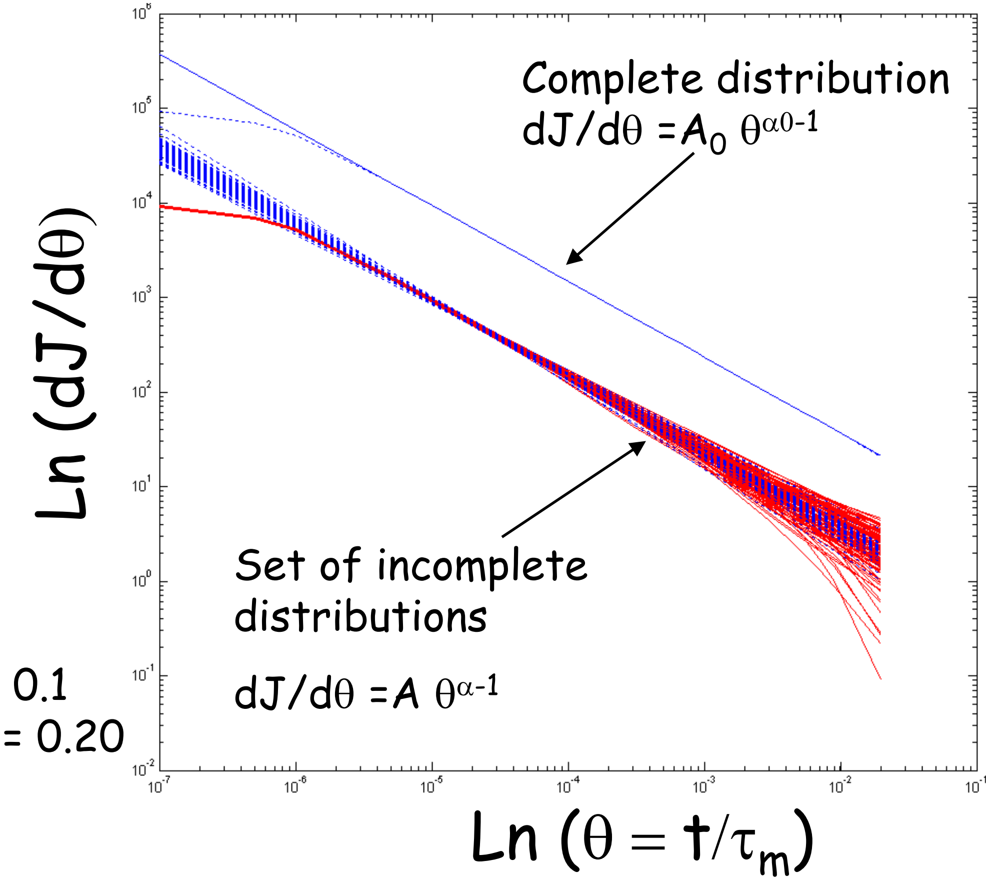
Response of the system



Complete distribution (idealized)

Incomplete distribution: we randomly keep a fraction s of the elements

(Simulates the variability from one cell to another)



s = 0.1
α_0 = 0.20

$dJ/d\theta = A\ \theta^{\alpha-1}$

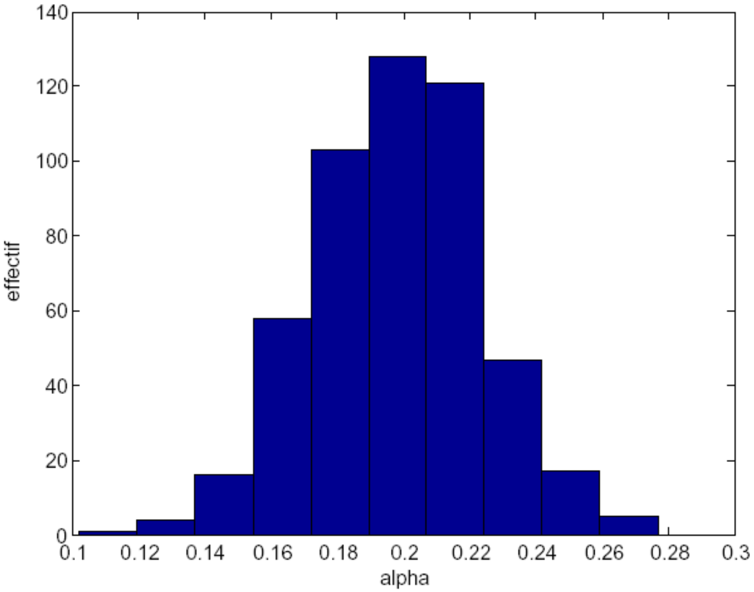
$J(\theta) = (A/\alpha)\ \theta^{\alpha}$

Dispersion of coefficients of the power law

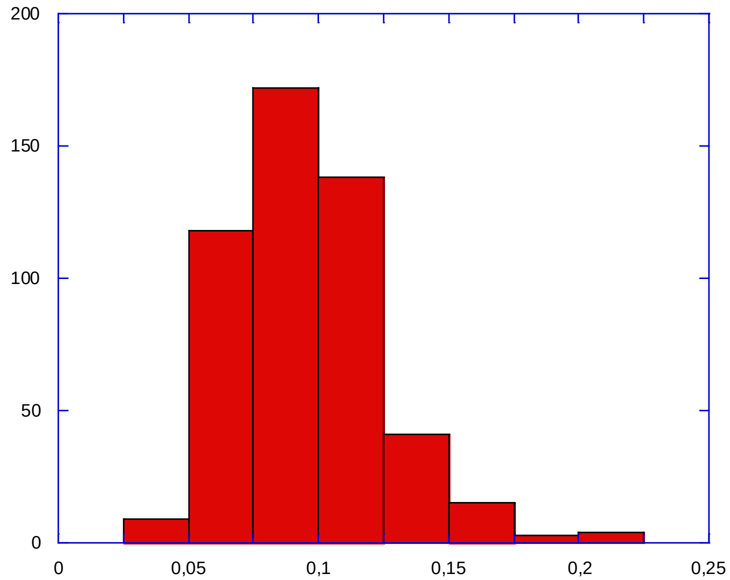
$J(\theta) = A\ \theta^{\alpha}$

- Normal distribution of exponents α
- Log-normale distribution of factors A

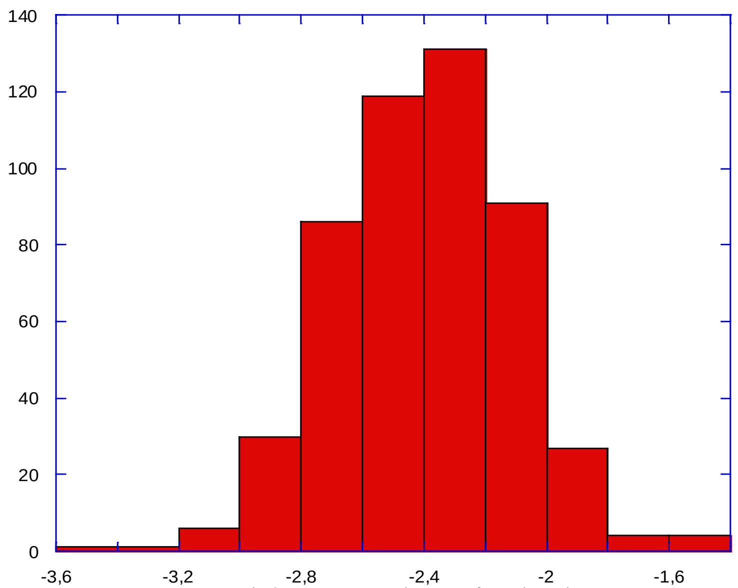
cf experimental results



Exponent α



Factor A



Ln (factor) = ln(A)

Soft Glassy Material or …

Like foams, emulsion, sluries

Desordered medium with a great number of elements and **out of equimibrium**

Interaction between mesoscopic elements leads to

→ large distributions of sizes and relaxation times:

no characteristic time scale

→ specific relaxation processes :

non viscous dissipation

Parameter of control **x (noise temperature)**

→ **power law** rheological behaviour, $\alpha = x - 1$

Possible origins of the power law behavior

foams, emulsions, pastes, slurries

- Out of equilibrium
- Permanent structural rearrangement

↓

Soft Glassy Materials (SGM)

Sollich, ***Phys. Rev. E*** (1998)

⇩

Dynamic origin

Partially polymerized gels

- Fixed structure
- Fractal dimension

↓

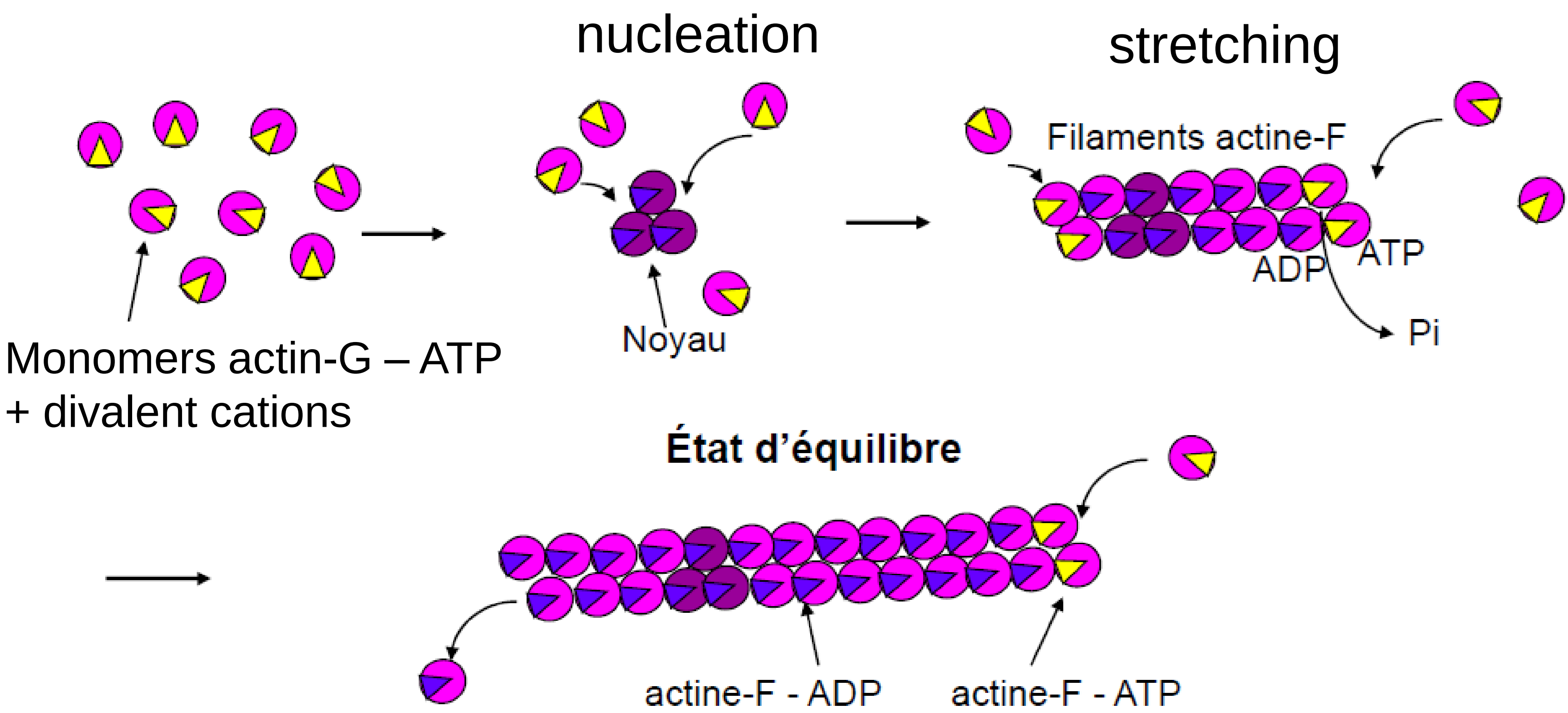
Materials at the « Sol-Gel » transition

Winter *et al.*, ***J. of Rheology*** (1986)

⇩

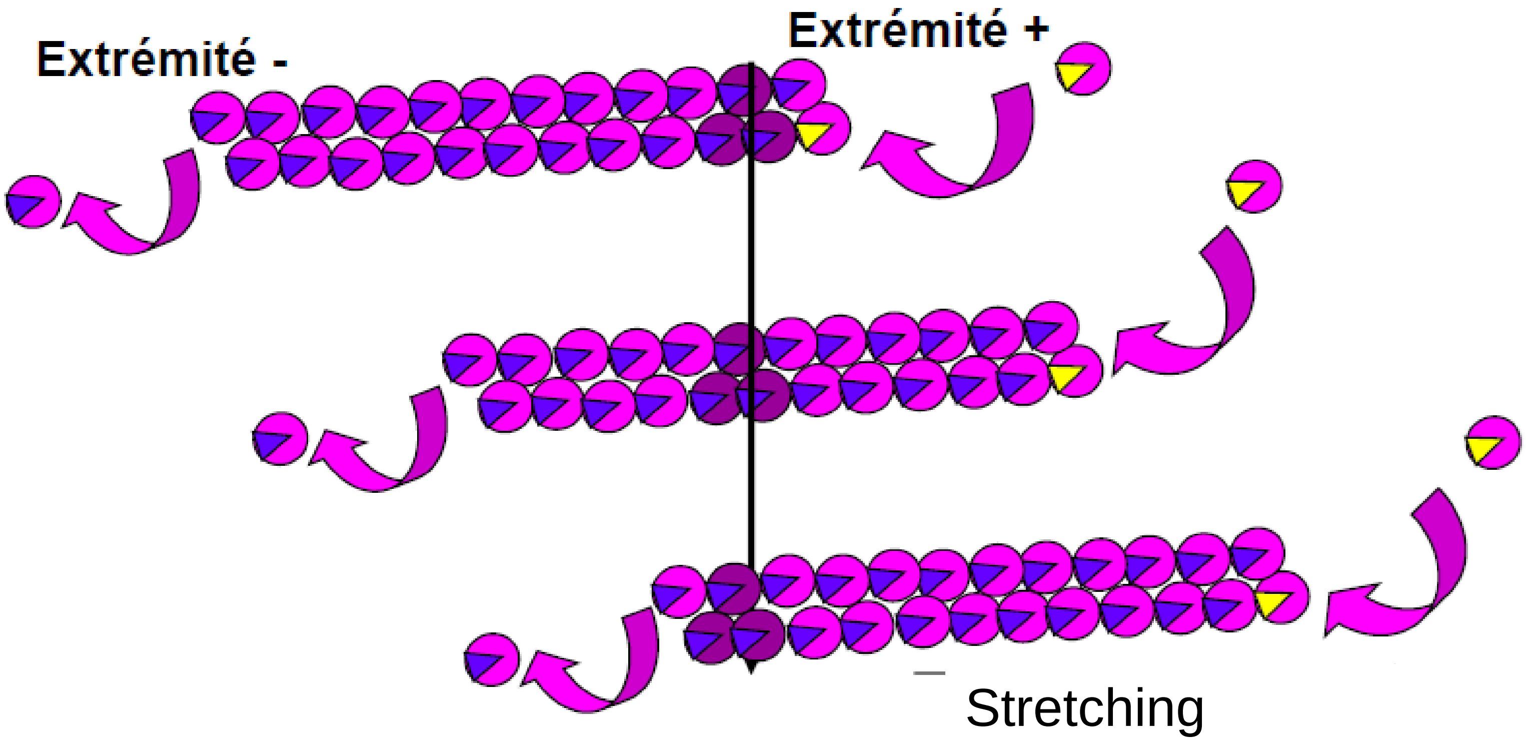
Structural origin

POLYMERIZATION OF ACTIN FILAMENTS

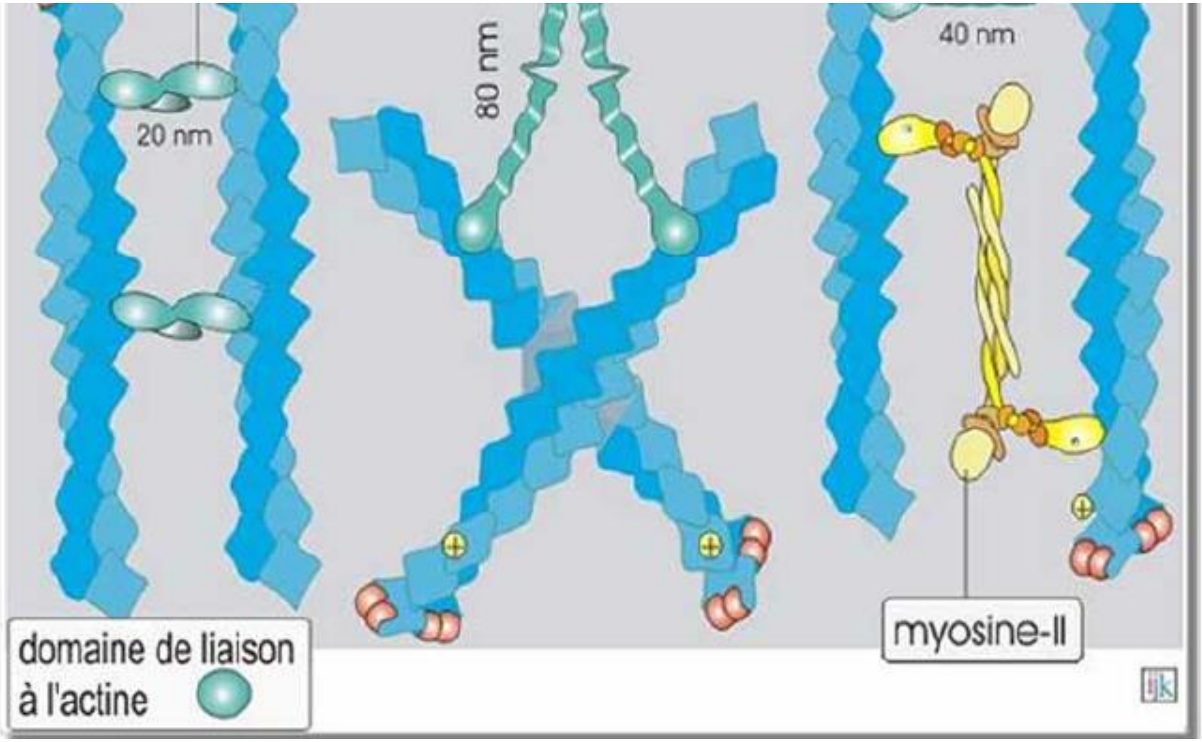


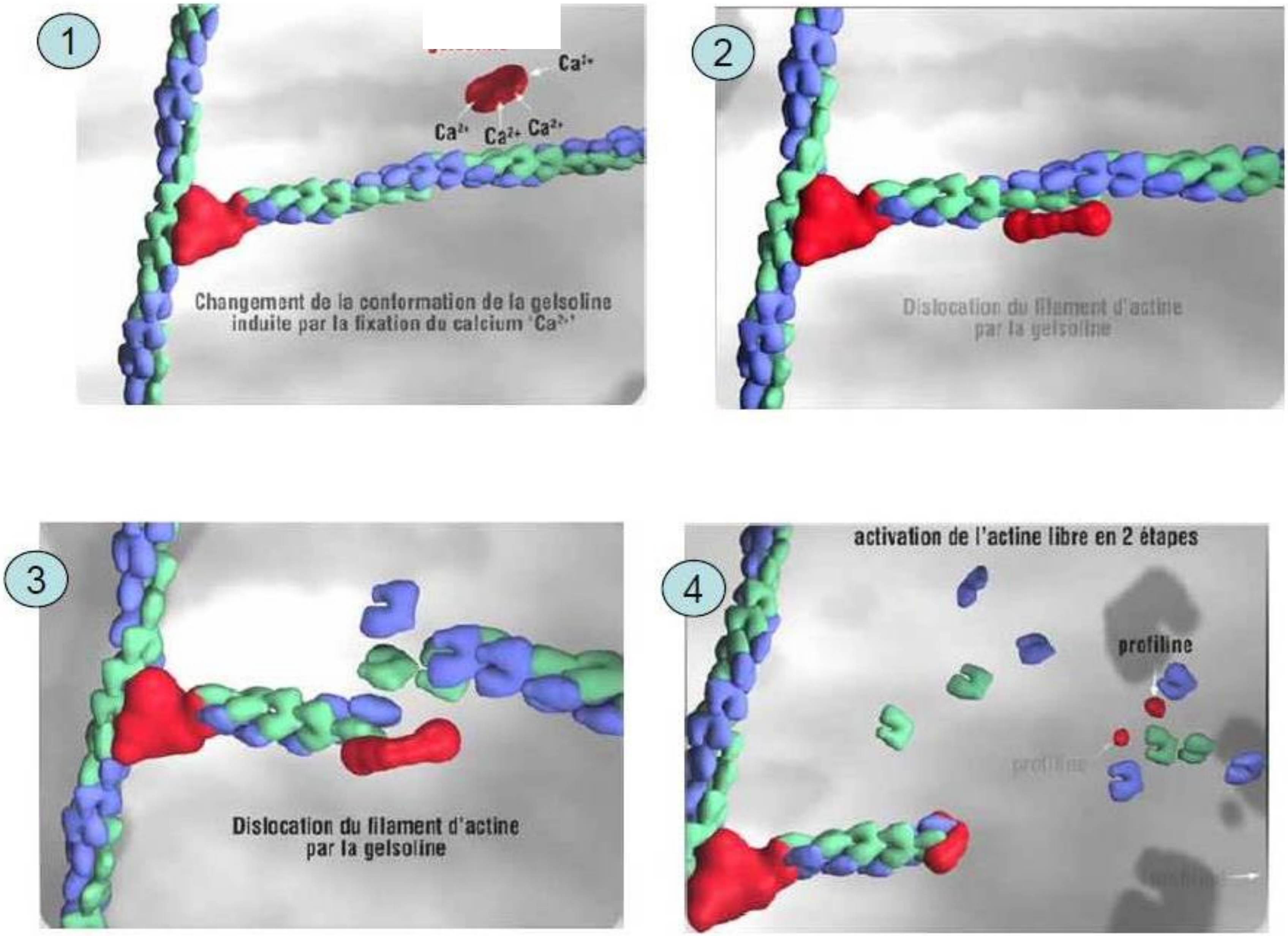
treadmilling

Cc- (0,8µM)> C actine-G > Cc+ (0,1µM)



STRUCTURE OF ACTIN FILAMENTS IN THE CELL




1
Ca²⁺
Ca²⁺ Ca²⁺ Ca²⁺
Changement de la conformation de la gelsoline
induite par la fixation du calcium 'Ca²⁺'
2
Dislocation du filament d'actine
par la gelsoline
3
Dislocation du filament d'actine
par la gelsoline
activation de l'actine libre en 2 étapes
4
profiline
profiline